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M. W. GREAVES, PRESIDENT, AND H. HÖNIGSMANN, SECRETARY

Morning Session, Monday, April 5th, 1982—*Presidential Address,* 8:30 AM—**First Session,** 8:35-10:40 AM—H. SCHAEFER, Chairman

Wavelength Specificity and Time Course of PUVA-induced Changes in Langerhans Cells. P. S. FRIEDMANN, G. FORD, J. ROSS*, AND B. DIFFEY**, Depts. of Dermatology, University of Newcastle upon Tyne and *Edinburgh Royal Infirmary, **Dept. of Medical Physics, Dryburn Hospital, Durham.

During PUVA therapy of psoriasis the numbers of Langerhans cells (LCs) are reduced from 720 ± 90/mm^2 to 60 ± 20 mm^2 after 7 exposures. The present study has examined the rate of recovery from this effect and the relationship to wavelength. Throughout the course of PUVA treatment LC numbers remain low. Electron microscopy confirms that they have disappeared and not simply lost ATPase activity: the ratio of LCs to basal keratinocytes was 0.05 before treatment and fell to 0.005 during PUVA. One week after cessation of PUVA there was no change, by 2 weeks they had returned to 50% and by 3 weeks they were back to 735 ± 35/mm^2. Since the emission of PUVA lamps contains traces of UV-B the separate effects of UV-A and B were tested. In the absence of 8-MOP neither UV-A alone (UV-B filtered out with Melinex) nor the unfiltered emission (UV-A+B) (3 J/cm^2/dose) caused any reduction in LC numbers. In the presence of 8-MOP, 5 exposures to monochromated UV-A (2 J/cm^2/dose) depleted LCs to 60/mm^2, whereas monochromated UV-B (10 mJ/cm^2/dose) caused a slight increase in LCs from 720 ± 90/mm^2 to 912 ± 80/mm^2. We examined the effects of UV-B further since our observation conflicted with those of Aberer. 5 doses (10 or 20 mJ/cm^2) of monochromated UV-B given on alternate days caused no depletion of LCs whereas 48 hr after a single dose of 2MED (100 ± 50 mJ/cm^2) or more, there was total loss of ATPase stained LCs. Impairment of LC function with UV-B or PUVA is a potentially useful way of manipulating the immune system. However, possible undesirable effects such as susceptibility to UV carcinogenesis seem likely to occur with doses of UV encountered on normal sunny days.

Physical Chemistry of Anthralin in Model Systems and Human Skin. T. SA E MELO, L. DUBERTRET°, P. PROGNON°°, A. GOND°°, G. MAHUZIER°°, R. SANTUS, MNHN—43, rue Cuvier—75231 Paris-France, °Hôpital Henri Mondor—94010 Creteil-France, °°Faculté de Pharmacie—92290 Chatenay-Malabry-France.

The physico-chemical properties and the stability of anthralin an antipsoriatic agent have been characterized and quantified by optical absorption, fluorescence and gaschromatography coupled to mass spectrometry (GCMS) in various media under controlled conditions with respect to the presence and absence of light and/or oxygen. Characterization of the anionic species in equilibrium with the neutral molecule in aqueous media was possible with anthralin and its oxidation product (1.8-dihydroxyanthraquinone) leading to pKa = 9.4 for both compounds. In contrast with the "anthralin dimer" (1-8, 1'-8' tetrahydroxydianthron) anthralin readily binds to human serum albumin at one major site with a binding constant of 2.3 × 10^5 M^{-1} leading to the anthralin anion which oxidizes yielding the 1.8-dihydroxyanthraquinone which in turn binds to HSA. Studies on the interaction of anthralin with DNA indicates that previously reported spectral shift cannot be attributed to complex formation. These results correlated with those obtained with intact whole human epidermis and suction blister fluid show that, in the former case, anthralin binds to skin proteins as demonstrated by absorption and fluorescence spectroscopy performed on skin which stabilizes the anthralin anion because of hindered oxygen diffusion in skin proteins. GCMS analysis makes it easy to detect anthralin and 1.8-dihydroxyanthraquinone in suction blister fluid doped with anthralin but not in blister fluid obtained after topical application on normal human skin although this technique can detect 10^{-11} M anthralin. This physico-chemical study suggests that the anthralin anion because of its binding capacity to proteins and oxygen sensitivity plays a key role in the therapeutic action of anthralin.

The *in vivo* Fate of Anthralin in the Skin of the Hairless Rat. DANIEL CAVEY, BRAHAM SHROOT, AND RONALD DICKINSON, Centre International de Recherches Dermatologiques Sophia Antipolis, 06565 Valbonne Cédex, France.

The mechanism of action of the topical antipsoriatic agent anthralin and the nature of the molecular species involved remain obscure. The aim of the present study was to follow the *in vivo* fate of anthralin in hairless rat skin by (1) quantitative analysis of anthralin and two of its degradation products (dimer and quinone) which can be recovered from the exposed skin by solvent extraction and (2) quantifying the amount of penetrated material which cannot be extracted. After topical application of anthralin (240 μg/cm^2) in the presence of ^{3}H-anthralin as a tracer, the exposed skin was extracted with di-isopropylether: the extractible material was analysed by HPLC and counted; the non-extractible material was solubilized and counted. With continuous application, anthralin appeared rapidly in the skin, reached a plateau of ≃ 1.3 μg/cm^2 (30 min-24 h), and was essentially located in the horny layer. No significant amounts of quinone were detected, whereas substantial dimer formation occurred (molar ratio: dimer/anthralin ≃ 0.5 after ≃ 24 h). The non-extractible fraction increased linearly with time (ratio: non-extracted ^{3}H/extracted ^{3}H ≃ 4 after 24 h) and was situated under the horny layer. Analysis of the skin at different times after a 1 h application showed that anthralin disappeared from the horny layer within ≃ 2 h and was not accounted for, either unchanged or as dimer or quinone, in the lower layers. Only half of the disappeared radioactivity was integrated into the nonextractible fraction. The role of the dimer, as well as the significance, nature and role of the nonextractible fraction are currently under investigation.

Effects of Aromatic Retinoic Treatment on Epidermal Langerhans Cells in Psoriasis. A Quantitative Study. M. HAFTEK, M. FAURE, D. SCHMITT, J. THIVOLET, INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot-Pav. R, 69374 Lyon Cedex 2.

Langerhans cells were reported to mediate delayed hypersensitivity reactions, but their role in epidermal growth control is also discussed. Both factors are of extreme importance in psoriasis, where number of LC appears to be perturbated. We have evaluated number and distribution of LC in psoriatic epidermis at various stages of the disease and under treatment with Tigason. Involved (IPS) and noninvolved (NIPS) skin of forearm extensory surface of 12 patients with psoriasis was biopsied before, at 1 or 3 wk; 3 or 4 wk and after clearing of lesions. ATPase staining method performed on EDTA-separated epidermal sheets was used in parallel with a quantitative IF method of LC enumeration on frozen skin sections using OKT6 and anti HLA-DR monoclonal antibodies. The latter technique allowed to observe not only the number and distribution of LC in epidermis, but also IF(+)/IF(−) cell ratio and presence of IF(+) cells in dermal capillaries and infiltrate. In well-developed psoriatic plaques before treatment the T6(+) dendritic cells were rare and appeared in small groups of 4-5 cells. HLA-DR(+) cells in same biopsies were much more numerous, what may reflect the presence of HLA-DR(+) infiltrate cells in IPS epidermis. In NIPS epidermis the T6(+)/HLA-DR(+) cell ratio was opposite than in IPS and similar to one characteristic for normal human epidermis. We propose, that OKT6 monoclonal antibody, specific for epidermal dendritic cells of LC phenotype, do not distinguish immunologically mature LC from their immature forms, not expressing HLA-DR antigen, necessary for antigen-presenting function. During treatment with Tigason the number of HLA-DR(+) cells in IPS gradually diminished, while T6(+) cells number and distribution returned to normal. Changes in keratinocytes/LC ratio and LD distribution during retinoid treatment will be discussed.

Comparison of 13-cis Retinoic Acid and Cyproterone Acetate on the Clinical Response, Sebum Secretion, Dermal and Epidermal Lipogenesis in Acne. F. LYONS, J. MARSDEN, AND SAM SHUSTER, Dept. of Dermatology, University of Newcastle upon Tyne. NE1 4LP.

Our evidence that increased sebum production is the major cause of adolescent acne conflicts with the more popular explanation of ductal obstruction by keratin. To help resolve this problem we have compared the clinical response with the degree of change in sebum excretion (SER) and dermal lipogenesis which is sebaceous in origin and epidermal lipogenesis which is related to "keratinisation" in epidermis and the upper parts of the follicular epithelium. Seventeen men with severe acne were studied; 10 were given 0.8 mg/kg daily 13-cis RA and 7 were given 100 mg/kg daily CPA each for 10 weeks. Clinical response was measured as pimple counts on the forehead and SER and lipogenesis were measured on punch biopsies as described elsewhere. The pimple counts fell from 54 ± 6 to 18 ± 3 after CPA and 48 ± 6 to 10 ± 2 after 13-cis RA. The corresponding SERs were 1.5 ± 0.1 falling to 0.5 ± 0.1 for CPA, 1.7 ± 0.1 falling to 0.2 ± 0.04 for 13-cis RA. The magnitude of the clinical improvement measured as pimple count corresponded in each subject to the change in SER. Sebaceous lipogenesis was decreased by both drugs. Although this decrease was greater with 13-cis RA the 2 sets of results could not be compared as for technical reasons the results were not satisfactory after CPA. By contrast epidermal lipogenesis was unchanged after CPA but increased after 13-cis RA from 4912 ± 400 to 6789 ± 700 dpm/4 mm punch biopsy ($P < 0.02$). Thus the response to the 2 different drugs occurred regardless of whether epidermal lipogenesis was increased or unchanged, the common feature being a decrease in sebum production, the degree of improvement being related to the magnitude of this decrease. Elsewhere we have shown the converse that the severity of acne corresponds to the degree of increase in SER. We therefore conclude that SER in the main causal defect in adolescent acne.

Lecture, 10:00-10:40 AM—I.L. BONTA: "Prostaglandins and Leukotrienes: Biological Relevance"

Second Session, 11:10 AM-1:00 PM-Ö HÄGERMARK, Chairman

A New Method for Continuous Measurement of Inflammatory Mediators Released from Epidermis and Dermis, Respectively. W. SCHALLA, A. CIVIER, C. HENSBY, L. JUHLIN, AND H. SCHAEFER. CIRD, Sophia Antipolis, Valbonne, France.

At present there are only very invasive techniques available to study the kinetics of mediators involved in skin inflammation. Therefore, we developed a less aggressive technique which differentiate in addition between epidermal and dermal mediator activity. A flat flow chamber (diameter:3 cm, height:5 mm) on

the lower forearm was perfused with a solution containing 0.2 mM $NADH_2$ and 0.7 mM pyruvate in Ringer carbonate buffer. After passing the chamber and filtering (Millipore:0.45 μm), the fluid was collected in fractions. For measuring the epidermal liberation, the horny layer was stripped before fixation of the chamber, for the dermal mediator release the epidermis was at first removed after using the suction blister technique for separation of the epidermis from the dermis. The $NADH_2$ consumption was measured in the fractions and then the prostaglandins were analysed after extraction and preparative TLC by quantitative GC-MS. After about 30 min the increased $NADH_2$ consumption and the increased amount of released PGD_2 and $PGF_{2\alpha}$ turned back to a steady state level indicating the end of the wash out period from damaged cells. Intraindividually the epidermal PGD_2 and $PGF_{2\alpha}$ release is higher than the dermal ones, the amount of PGD_2 released is somewhat higher than the $PGF_{2\alpha}$ values. Nevertheless, all non stimulated PGD_2 and $PGF_{2\alpha}$ amounts released lie around or somewhat higher than 1 $ng \cdot cm^{-2}$ per 5 to 10 min fractions. The results obtained are promising and indicate that this method is not only applicable to prostaglandin liberation kinetics, but also to those of other mediators and substances and their pharmacological responses to different stimuli, agonists and antagonists.

Raised Arachidonic Acid and Prostaglandin Levels in Dithranol Erythema: Time Course Study. R. Barr, K. J. Misch, C. Hensby*, A. I. Mallet, and M. W. Greaves. Institute of Dermatology, Homerton Grove, London E9 6BX; *Centre International de Recherches Dermatologiques, Sophia-Antipolis, 06560 Valbonne, France.

The principal limitation to dithranol (anthralin) treatment of chronic plaque psoriasis is local irritation and burning. The purpose of this study is to investigate the pharmacological mechanism of these reactions. Dithranol (0.25–1% w/w) in yellow soft paraffin was applied for up to 24 hr to the uninvolved abdominal skin of 6 patients with chronic plaque psoriasis. Using the suction bulla technique blister fluid was obtained from inflamed skin at 6, 12, 24, 48, 72, and 96 and 168 hr and from control skin. Each 100 μl blister fluid sample was equilibrated with deuterated internal standards. Arachidonic acid and prostaglandins (PG) were extracted with ethyl acetate, separated by high pressure liquid chromatography and analysed by gas chromatography-mass spectrometry. Erythema developed by 6–12 hr and was maximal by 48 hr. It persisted maximally to 72 hr then slowly diminishing to be barely detectable at 168 hr. Arachidonic acid levels increased from 2.7 μg/ml in control exudates to 5.3 μg/ml at 48 hr and then slowly declined. PGE_2 and $PGF_{2\alpha}$ concentrations increased from 26 and 9 ng/ml to 93 and 16 ng/ml at 12 hr respectively but rapidly fell to control values by 48 hr. There was a significant correlation between PGE_2 and $PGF_{2\alpha}$ levels in the exudates. Concentrations of PGD_2 and 6-oxo-$PGF_{1\alpha}$ (the stable metabolite of prostacyclin) showed no significant change. The results indicate an association between arachidonic acid, PGE_2 and $F_{2\alpha}$ but not prostacyclin or PGD_2 and the development of dithranol inflammation. While PGE_2 and $F_{2\alpha}$ fall rapidly to control values, arachidonic acid levels more closely parallel the erythema time course. The role of these compounds remain to be established.

The Effect of Heat Due to Infra Red (IR) Irradiation on Human Skin Prostaglandin Concentrations. L. Juhlin, A. Civier, C. N. Hensby, and S. Shroot. CIRD, Sophia Antipolis, Valbonne, France.

The pharmacological effect of heat producing, long wavelengths (IR) to which skin is often exposed, have not been well defined. Heat is often used in the treatment of inflammatory disorders and of particular relevance is its recent use in the treatment of psoriasis. We have therefore studied the effect of heat due to IR irradiation on skin prostaglandin concentrations. Forearms were irradiated with an Osram theratherm 250W IR lamp, placed 43 cms above the skin. Exudate was obtained from irradiated and non-irradiated skin using a suction bullae technique during, immediately after and 4–5 h after irradiation. The exudates after extraction and preparative TLC were quantitatively analysed for PGD_2, $F_{2\alpha}$ and 6-oxo-$F_{1\alpha}$ by GC-MS. The results ($ngml^{-1}$, mean SEM) are summarised in the table. The skin surface temperature increased during irradiation (1h) from 28–30°C to 35–37°C. These elevations, due to IR, irradiation suggest that care must be taken when using experimental and clinical techniques that may increase the temperature in association with mediator studies.

	n	PGD_2	$PGF_{2\alpha}$	6-oxo-$PGF_{1\alpha}$
Non-irrad. controls	8	11.1 ±1.0	12.2 ±1.2	7.2 ±1.7
Bullae formed during irrad.	7	33.9*** ±4.9	29.8*** ±3.3	29.9*** ±4.0
Irrad.(1h) after form. of bullae	4	19.2** ±2.2	22.5 ±1.3	13.0 ±4.4
Bullae raised and sampled 4–5 h after irradiation	4	38.0*** ±5.6	40.6*** ±4.6	40.7*** ±3.8

**$p > 0.01 ***$p > 0.001$

The Synthesis of Leukotriene B_4-like Material by Cultured Human Keratinocytes. S. D. Brain, [1]R. D. R. Camp, [2]I. M. Leigh, and [3]A. W. Ford-Hutchinson, [1]Institute of Dermatology, London E9 6BX; [2]Imperial Cancer Research Fund, London WC2A 3PX; [3]King's College Hospital Medical School, London SE5.

The arachidonate lipoxygenase product leukotriene B_4 (LTB_4) is a potent neutrophil chemokinetic and chemotactic substance (Ford-Hutchinson et al, *Nature 286*, 264, 1980), with a potency similar to that of C5a. It is also capable of inducing accumulation of neutrophils on injection into human skin. We have investigated the ability of cultured human keratinocytes to form LTB_4. Keratinocytes were obtained from human mastectomy specimens and were cultured with 3T3 fibroblast feeder cells in RPMI 1640 medium supplemented with hydrocortisone and cholera toxin. The hydrocortisone was removed 24h before the confluent keratinocytes were resuspended in modified essential medium (Eagle) buffered to pH 7.4 with HEPES, at a concentration of 10^6 cells/ml. The suspensions were incubated for 4 min at 37°C with or without calcium ionophore A23187. The mixture was extracted and purified by silicic acid chromatography as described by Borgeat and Samuelsson (*Proc Natl Acad Sci* USA 76: 2148, 1979). The polar fraction was subjected to reversed phase HPLC using methanol/water/acetic acid (72:28:0.01). Fractions of 1 min were collected and tested for chemokinetic activity towards human neutrophils in an agarose microdroplet bioassay (Smith & Walker, *Br J Pharmac* 69: 473, 1980). Similar experiments using 3T3 fibroblasts were carried out. In each keratinocyte experiment a significant single peak of chemokinetic activity was found in HPLC fractions co-eluting with standard LTB_4. Approximately 15–40 ng LTB_4 equivalents were released per 10^8 ionophore-stimulated keratinocytes. In contrast unstimulated keratinocytes released significantly less LTB_4-like material, and 3T3 fibroblasts produced no detectable chemokinetic activity. These findings strongly suggest that human keratinocytes are capable of releasing LTB_4, which may play a role in the recruitment of neutrophils to sites of skin inflammation.

Lecture, 12:15–1:00 PM—B. Czarnetzki: "Pharmacology of the Eosinophil"

Workshops, 1:00–3:00 PM. (1) Pharmacology of the Mast Cell, S.I. Wasserman and M.W. Greaves. (2) Drugs influencing DNA Repair, S. Spadari and R. Waters. (3) Food Intolerance in Skin Disease, H. Doeglas and J. Brostoff. (4) New Trends in Dermatological Genetics, R. Happle and I. Anton-Lamprecht.

Afternoon Session, 3:00–6:00 PM—*Poster Viewing*, 3:00–4:30 PM

Is There a Role For Bacteria in the Imitiation of Acne Vulgaris? J. P. Leeming, K. T. Holland, and *W. J. Cunliffe, University Departments of Microbiology and *Dermatology, Leeds, U. K.

It is widely believed that the comedone is the first morphological stage in the development of a normal follicle into an acne lesion. Cutaneous bacteria have frequently been described as major components of comedones and have been implicated in the pathogenesis of acne vulgaris. However, comprehensive numerical comparisons of the flora of individual normal follicles and comedones have not been reported. In this investigation the bacterial flora of apparently normal follicles and comedones have been studied both qualitatively and quantitatively after microdissection from biopsies of skin from the upper backs of acne patients. A microdissection procedure involving pretreatment in 1 M-calcium chloride was followed after performing suitable control experiments to ensure that the procedure did not result in loss of bacterial viability. Follicles were processed individually and numbers and types of bacterial inhabitants were determined. The limit of sensitivity was thirteen bacteria per follicle.

The predominant bacterial groups in all colonised follicles were staphylococci and propionibacteria. A large proportion of normal follicles contained no bacteria; those colonised contained up to 10^4 staphylococci and up to 10^7 propionibacteria. A small number of comedones contained no bacteria, however a relatively large proportion contained either no staphylococci or no propionibacteria. Those colonised contained up to 10^6 staphylococci and up to 10^7 propionibacteria. It is concluded from the data obtained that bacterial colonisation of a follicle is not a prerequisite for comedone formation.

Physiological Responses of Cutaneous Propionibacteria to Changes in Dissolved Oxygen Concentration. *J. H. Cove, *K. T. Holland, and W. J. Cunliffe**, *Department of Microbiology, University of Leeds and **Department of Dermatology, The General Infirmary, Leeds, U.K.

Skin bacteria produce a wide range of exocellular products which interact with the different components of their environment and with the host immune system which occasionally leads to inflammatory acne. The production of exoenzymes by these bacteria responds to environmental change such as pH and oxygen tension. It is likely that cutaneous bacteria are exposed to changes in the dissolved oxygen concentration of their microenvironment both as a result of normal and disease processes. It has been established that the 3 species of cutaneous propionibacteria are capable of growth and exoenzyme production in the presence of oxygen although these organisms were described previously as anaerobes. The object of this study was to further investigate the physiological response of cutaneous propionibacteria to changes in dissolved oxygen concentration. *Propionibacterium acnes*, *Propionibacterium granulosum* and *Propionibacterium avidum* were grown using continuous culture techniques which control precisely environmental parameters such as nutrient supply, temperature, pH and dissolved oxygen concentration. Three aspects of the study are reported. (1) Factors related to growth and exoenzyme production such as the levels of total protein, NH_4^+, PO_4^{3-}, fermentation end products and sugars were measured in steady state cultures growing anaerobically and in the presence of oxygen. (2) The viability of bacteria growing aerobically and in transition from anaerobic to aerobic conditions was determined. (3) The stability of propionibacterial exoenzymes was compared under aerobic and anaerobic conditions. The results show that cutaneous propionibacteria are well adapted to survive environments with a changing dissolved oxygen concentration. Therefore it is not necessary to consider the microenvironment of the follicle as anaerobic for these bacteria to persist and grow.

The Effect of 13-cis-Retinoic Acid on the Skin Microflora of Patients with Severe Acne. Kathrine King, D. H. Jones, Diana Daltrey, and W. J. Cunliffe, School of Health and Applied Sciences, Leeds Polytechnic; University Departments of Dermatology and Immunology, Leeds, U. K.

Previous studies have shown systemic 13-cis-retinoic acid to be effective in the treatment of severe acne. Therapy is associated with a marked reduction in the sebum excretion rate. Microbial populations may also be of importance in acne. The numbers of skin microorganisms and production rate of free fatty acids (FFA) were estimated in 48 patients before, during and after treatment with 13-cis-retinoic acid, at 3 dosage levels, as part of a clinical and laboratory dose response study. Treatment caused a significant reduction in all components of the skin microflora (at least one log cycle); decrease was greatest in the density of propionibacteria (2 or more log cycles). After treatment was terminated all counts gradually rose. However, the numbers of microorganisms present after a follow-up period of four months were significantly lower than the numbers present before treatment began, with the exception of the anaerobic populations of low-dose patients. The production rate of FFA (a measure of microbial function) was significantly reduced (58–75%) after 4 weeks therapy. The reduced level was maintained until cessation of treatment when the production rate increased. At the end of trial levels were significantly lower than initially; changes in FFA production rate were not dose dependent. These changes in bacterial numbers and function further help to explain the mechanisms of action of the drug.

Studies of the Exocellular Proteolytic Activity Produced by Propionibacterium Acnes. Eileen Ingham*, K. T. Holland+, G. Gowland*, and W. J. Cunliffe**, University Departments of Immunology*, Microbiology+ and Dermatology**, Leeds, U. K.

P. acnes and/or its products have been implicated in the initiation and persistence of inflammation in acne. Protease, potentially the most important activity with regard to complement activation and/or release of chemotactic substances *in vivo*, has been neglected. Low levels of protease were obtained in laboratory media with *P. acnes* (strain P-37). Maximum production occurred in

Trypticase Soya Broth (TSB; Gibco) + D-sorbitol (0.4% w/v). Batch and continuous culture studies showed that protease production was associated with exponential growth or high specific growth rate. Protease produced during the exponential phase of growth of P-37 in TSB + 0.4% (w/v) D-sorbitol was partially purified by 70% $(NH_4)_2SO_4$ pp[m]; G-75 chromatography and DEAE-Sephadex A-50 ion exchange. Protease was composed of 3 species of enzyme which were separated by the latter procedure. These were (i) a serine protease, mol. wt. 20–30,000, pH optimum 6.5–7.5, inactivated by EDTA (10^{-3} M), cationic at pH 7; (ii) an alkaline protease, mol. wt. 30–40,000, pH optimum 7–9, anionic at pH 7 and (iii) a Ca^{2+} stimulated protease, mol. wt. 20–30,000, pH optimum 7–9, inactivated by EDTA (10^{-4} M), anionic at pH 7. These proteases have relatively low mol. wts. and may diffuse to and into the follicular wall causing subtle loss of integrity, promoting diffusion of themselves and larger molecules to the follicular dermal interface where the initiation of inflammation occurs. It is interesting to contemplate why the proteolytic activity is maximal above pH 7.

Cimetidine as an H_1-Agonist: Increase of Intracellular cGMP and Modification of "Early" E-Rosettes Percentage. A. M. Castellazzi*, F. Serri**, J. W. Hadden***, A. Giannetti*, Department of Dermatology, University of Pavia*, Italy, Department of Dermatology, "Sacro Cuore" University**, Rome, Italy, Department of Immunopharmacology, Sloan-Kettering Institute***, New York, N.Y., U.S.A.

Several authors have found Cimetidine to be an H_2-receptor blocker. Some evidence in which Cimetidine (Cim.) may induce an immunostimulatory lymphokine and an increase of lymphoproliferative response suggested that Cim. may be an H_1-agonist in addition to being an H_2-antagonist. In attempt to confirm this hypothesis we carried out the following experiments: (1) The dose-response curve, using doses of Cim. from 10^{-9} M to 10^{-3} M, looking for the most effective dose in the 2 models used. (2) The time-course of increase in intracellular cGMP after Cim. addition (H_1-linked event). (3) The variation in percent of "Early" E-Rosettes (cGMP-increase-linked event). The dose response curve showed that Cim. 10^{-6} M was the most effective dose in both models. At this dose the cGMP level in lymphocytes at 10 min increased from 343 ± 141 fm/mg prot. in the control to 604 ± 115 fm/mg prot. after Cim. (Average of 7 experimts: $p < 0.001$). At the same dose the percentage of "Early" E-Rosettes increased from 34.2 ± 5 in the control to 54.2 ± 9 after Cim. (Average of 7 experiments: $p < 0.001$ n Student's t-test). These data suggest that Cim. directly increases intracellular cGMP level and is active in a function cGMP-increase-linked. Thus Cim. can be considered an H_1 agonist.

β-Adrenergic and Histamine Receptors in Cultured Skin Fibroblasts and Keratinocytes. J. Gazith, M. T. Cavey, P. P. Elena, B. Shroot, and U. Reichert. Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne Cédex, France.

The existence of β-adrenergic and histamine receptors in skin has been suggested by, and related to, observations *in vivo* of patients with psoriasis or skin inflammation. The present study was undertaken in order to demonstrate and characterize these membrane receptors in terms of affinity, specificity, and receptor density. Cultured human keratinocytes were chosen as specific epidermal cells and skin fibroblasts as typical dermal cells. Binding studies, using tritium labeled antagonists with high specific activities were carried out on membrane fractions of the cells. High speed filtration on glass fiber filters was employed to separate the bound from free ligands. *1. β-adrenergic receptors:* Keratinocyte membranes show a high density of these receptors when compared with tissues such as cerebellum, cerebral cortex, spleen or lung (270 fmol/mg protein). The dissociation constant (K_d) was found to be in the low nanomolar range, when using saturation binding experiments. For β-adrenergic agonists, using displacement studies, a lower affinity was found with a K_d in the micromolar range, but with no change in the receptor density. These have been confirmed using kinetic experiments to determine the association and dissociation rate constants. Using fibroblast membranes, a somewhat lower affinity (higher K_d) has been found for antagonists, ranging from 10 to 20 nanomolar, while the receptor density is lower when expressed on a basis of membrane protein (~100 fmol/mg protein). *2. Histamine receptors:* Using tritium labeled histamine H antagonists (doxepin and pyrilamine), high affinity binding of the antagonists to the membrane fraction from cultured human keratinocytes could be shown. This binding is characterized by high affinity (K_d of 5–15 nanomolar) and higher receptor density than that observed with the β-adrenergic receptors (0.7 nmol/mg protein).

Histamine and Non-Histamine Pharmacological Activity in Cold Urticaria. K. J. Misch, A. K. Black, R. Barr, C. N. Hensby*, A. I. Mallet, and M. W. Greaves. Institute of Dermatology, Homerton Grove, London E9 6BX; *Centre International de Recherches Dermatologiques, 06560 Valbonne, France.

As histamine suppression produces only incomplete clinical improvement in patients with cold urticaria, the presence of non-histaminic mediators was sought. 9 patients with primary acquired cold contact urticaria were studied. Venous blood was obtained before and after cold challenge to the forearm. Skin exudate was obtained by the suction bulla technique before and after cold challenge to the abdomen. Pharmacological activity was detected by superfusion bioassay cascade. Skin exudates were also analysed for arachidonic acid and prostaglandins (PG), after separation by high pressure liquid chromatography, by gas chromatography-mass spectrometry. Histamine release occurred in blood and skin exudate in parallel with development of cold-evoked wealing. It was 4.4 times higher in the skin exudate than in the venous blood. Elevated non-histaminic activity was detected in the exudate of 8 patients and the blood of 5. The differential activity raised the possibility of prostaglandin-like, bradykinin-like as well as other unidentified agents. Gas chromatography-mass spectrometry revealed no significant change in the mean arachidonic acid, PGE_2, PGD_2 and 6-oxo-$PGF_{1\alpha}$ (the stable breakdown product of prostacyclin) levels. However in skin exudate samples from 2 patients, both of whom showed elevated non-histaminic smooth muscle contracting activity, a rise in the concentration of PGE_2 occurred (30 ng/ml before cold challenge and 430 ng/ml after challenge, patient 1; 20 ng/ml before and 82 ng/ml after, patient 2. Arachidonic acid levels also increased in patient 1 from 3.6 to 5.0 μg/ml. Non-histaminic activity has been demonstrated in cold urticaria and this may explain the lack of effect of histamine suppression clinically. Prostaglandin E_2 may play a role in a small subgroup. The nature of the other non-histaminic activity remains to be elucidated.

Polyamines in Human Skin: Effects of Oral Retinoid Treatment. P. Elbaze[1], G. Milano[2], J. P. Ortonne[1]. [1]Service de Dermatologie, CHU Nice, France (Dr. Barety), and [2]Centre A. Lacassagne, Nice, France (Prof. Lalanne, Director).

The polyamines (PA) putrescine (PU), spermidine (SPD) and spermine (SM) can be considered intracellular regulators and extracellular indicators of cell kinetics. Free (F) and total (T) PA were studied in suction blister fluid (SB) obtained by the technique of Mustakallio, and in both the pure epidermis (E) forming the roof of the blister and the dermis (D). PA in 24-hr urines and serum were also evaluated. PA measurements were effected with a KONTRON LIQUIMAT III automatic amino acid analyzer. In a static study of 15 healthy persons, average PA values (nmoles/g) in E differed from those in D: PU-70 (E) vs 200 (D); SPD-270 (E) vs 130 (D); SM-860 (E) vs 200 (D). Based on the F/T and SPD/SM ratios, PA in SB are noticeably different from serum or urinary values, and are closer to PA distributions in D. In 5 patients with psoriasis and 6 other with various retinoidosensitive pathologies, PA were measured before and after 15 days of continuous treatment with etretinate Ro. 10.9359 (1 mg/kg/day per os). Among the results obtained, PA (T) variations in uninvolved E were: 15% drop in PU; 40% increase in SPD and 25% rise in SM. PA measurements in total skin must now thus be interpreted with caution in view of the respective concentrations in E and D. SB is an original fluid for PA evaluation. The effect of Ro. 10.9359 on PA metabolism in vivo is probably more complex than the mere inhibition of ornithine decarboxylase already described *in vitro* and *in vivo*.

The Relationship of Arginase Activity and Polyamine Levels in Psoriasis. J. Lauharanta (*) and K. Käpyaho (**). (*) Dept. of Dermatology, University Central Hospital, Helsinki, Finland, (**) Dept. of Biochemistry, University of Helsinki.

Polyamine biosynthesis is enhanced in psoriasis. Ornithine is a substrate for polyamine biosynthesis and would be depleted without compensatory supply. Arginase cleaves arginine to ornithine and urea. The aim of our study was to investigate the relationship of arginase activity and polyamine levels in psoriasis. Biopsy specimens from a psoriatic lesion and from the uninvolved skin of 6 psoriatics, and from the skin of 5 healthy controls were studied. Polyamines were determined after homogenization in 0.2 N perchloric acid as their fluorescent dansyl derivatives. Arginase activity was assayed by quantitating the conversion of radioactive arginine into ornithine which was separated by paper electrophoresis and counted for radioactivity by a liquid scintillation counter. The polyamine levels (nmol/mg DNA; mean ± SEM) were 41 ± 3 (putrescine), 323 ± 38 (spermidine) and 283 ± 15 (spermine) in involved psoriatic skin, 27 ± 3, 135 ± 13 and 226 ± 18 in uninvolved psoriatic skin, and 28 ± 3, 123 ± 15 and 229 ± 39 in control skin, respectively. The arginase activity (nmol ornithine/mg protein/h) was 277 ± 36 in involved psoriatic skin, 183 ± 58 in uninvolved psoriatic skin and 147 ± 9 in controls. Thus, the polyamine levels and the arginase activity were markedly higher in involved versus uninvolved psoriatic skin, whereas there was only a slight difference between the uninvolved psoriatic skin and control skin. In conclusion, arginase activity shows correlation with polyamine levels in psoriasis, apparently supplying the ornithine needed for enhanced polyamine biosynthesis.

Responses of Human Skin to Intradermal Injection of Leukotrienes C_4, D_4, and B_4. R. D. R. Camp, A. A. Coutts, M. W. Greaves, A. B. Kay[1] and M. J. Walport[1], Institute of Dermatology, Homerton Grove, London E9 6BX and [1]Department of Clinical Immunology, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

Leukotrienes (LTs) C_4, D_4, and B_4 are proposed as mediators of inflammation but little is known about their effects in human skin. Chemically synthesised LTC_4 and LTD_4 were supplied by J Rokach[1]; LTB_4 was prepared by biosynthesis[2]. The following were measured in up to 6 volunteers using a wide dose range of each agent: area of erythema, mean weal diameter, sensation, synergism with prostaglandin E_2 (PGE_2) and histological effects. LTC_4 and LTD_4 caused dose-related erythema and wealing, as little as 0.047 nmoles of each causing an erythemal response approximately half that of simultaneously injected histamine (1.63 nmoles). The ED50 for weal formation was 0.024 nmoles (LTC_4) and 0.035 nmoles (LTD_4). LTB_4 0.15–1.5 nmoles evoked an immediate erythemal reaction (without wealing) which was not dose-related. However, an area of induration developed at 30 min which was ill-defined, reaching a maximum diameter of 8 mm 60–120 min after injection, and persisting for at least 4 h. Pain and itch were minor and inconsistent with all LTs studied. Histological examination of LTB_4-evoked induration showed a pronounced dermal polymorphonuclear infiltrate with eosinophils and neutrophils. Similar biopsies from LTC_4 and LTD_4 injected sites showed no specific changes. Synergism was not demonstrated following simultaneous injection of PGE_2 0.14 nmoles with LTC_4, LTD_4 or LTB_4. These results support the possibility that LTC_4, LTD_4 and LTB_4. These results support the possibility that LTC_4, LTD_4 and LTB_4 are mediators of cutaneous inflammation.

The Production by Human Leucocytes of a Trihydroxyeicosatetraenoic Acid with Neutrophil Aggregating and Chemokinetic Properties. R. D. R. Camp, N. J. Fincham, A. W. Ford-Hutchinson*, A. I. Mallet, and P. M. Woollard, Institute of Dermatology, London E9 and *Department of Chemical Pathology, King's College Hospital Medical School, London SE5.

Several biologically active arachidonate lipoxygenase products have recently been shown to be synthesised by leucocytes. These products included leukotrienes B_4 (LTB_4), a potent chemokinetic and chemotactic compound. We now describe the formation of a novel trihydroxylated metabolite of arachidonic acid by human leucocytes and report its biological activity. Mixed human leucocytes, prepared from fresh blood by dextran sedimentation and centrifugation (Cunningham et al, *Br J Pharmacol* 63:119, 1978) were suspended in modified essential medium (Eagle) buffered to pH 7.4 with HEPES at a concentration of 3.5×10^7 cells/ml. This suspension was incubated with 0.11 mM arachidonic acid and 20 μM calcium ionophore A23187 for 4 min at 37°C. The mixture was extracted and purified by silicic acid chromatography (Borgeat & Samuelsson, *Proc Natl Acad Sci USA*, 76:2184, 1979) and the polar fraction subjected to reversed phase HPLC. A major UV absorbing compound more polar than LTB_4 was detected which exhibited a UV absorbance spectrum identical to that of LTB_4 (λmax 260, 270 and 281 nm). Analysis of the compound by gas chromatography-mass spectrometry indicated that it was a C-20 tetraunsaturated fatty acid hydroxylated at C-5 and C-12. The third hydroxyl group was assigned at C-20 and the structure was determined to be 5,12,20 trihydroxy 6,8,10,14-eicosatetraenoic acid. The effects of the compound on the chemokinesis and aggregation of neutrophils (Smith & Walker, *Br J Pharmacol* 69:473, 1980; Cunningham et al, *J Pharm Pharmacol* 22:377, 1980) were determined. Its activity was less than of LTB_4, but greater than that of the monohydroxylated metabolites of arachidonic acid. Our data suggest that the 5,12,20 trihydroxyeicosatetraenoic acid may be a metabolite of LTB_4 and may play a role as a mediator of inflammation.

Elevated Levels of Prostaglandins D_2 and $F_{2\alpha}$ in Suction Blister Fluid from Psoriatic Lesions. *C. Hensby, B. Berne, *A. Civier, *L. Juhlin, G.

[1] Rokach J, *et al:* Tetrahedron Letters, 21:1485, 1980

[2] Borgeat P, Samuelsson B: (1979) Proc Natl Acad Sci USA 76:2148, 1919

MICHAELSSON, C. VAHLQUIST. *CIRD, Sophia Antipolis, Valbonne, France. Department of Dermatology, University Hospital, Uppsala, Sweden.

Various inflammatory stimuli (Trafuril; UV-B) increase the level of prostaglandins in human skin. The purpose of our study was to determine the involvement of prostaglandins in chronic inflammation of human skin, exemplified by various types of psoriatic lesions. Psoriatic patients immediately before local or systemic chemotherapy were used for this study. Suction blisters were raised on psoriatic lesions and normal appearing skin. The exudates after extraction and preparative TLC were quantitatively analysed for PGD_2 and $PGF_{2\alpha}$ by GC-MS. The results (ng ml^{-1}) are given in the Table. They support the involvement of prostaglandins in the pathogenesis of psoriasis.

	PGD_2	$PGF_{2\alpha}$
Healthy volunteers (n = 8)	11.1 ±0.9	12.2 ±1.2
Psoriatic uninvolved (n = 9)	12.6 ±0.9	12.5 ±0.9
Psoriatic lesions (n = 10)	39.5*** ±3.4	44.1*** ±3.0

*** $p < 0.001$ relative to uninvolved.

Identification of a Thymopoietin-like Substance in Epidermal Cells from Normal Human Skin. A. C. CHU, G. GOLDSTEIN, C. BERGER, J. PATTERSON, R. EDELSON, St. John's Hospital, London., Columbia Presbyterian Hospital, New York and Ortho Pharmaceuticals, New Jersey.

An indirect immunofluorescence technique has been employed to demonstrate the presence of a thymopoietin (TP)-like substance in normal human epidermal cells. Cytocentrifuge smears of epidermal cells freshly trypsinised from normal skin, were fixed in 5% acetic acid in methanol and stained with a specific rabbit anti TP antibody. 8–14% (mean 10%) of the cells showed bright cytoplasmic fluorescence with the anti TP antibody. Controls using fibroblast suspensions in place of epidermal cells and non immune rabbit serum or rabbit anti ubiquitin in place of the anti TP antibody showed no positively labelled cells. Double labeling studies using OKT6 (a mouse monoclonal antibody reactive with Langerhans cells) and the anti TP showed that these cells have not Langerhans cells. Using epidermal cells harvested from 2–8 week old epidermal cell cultures showed that 2–5% of these cells still stained strongly with the anti TP antibody. This was found even when all exogenous sources of TP (i.e. serum) were excluded from the growth medium. *In situ* studies on 4 mm cryostat sections of normal human skin showed that the cells possessing this TP-like substance were the basal keratinocytes.

Post-Translational Phosphorylation of Keratin Gene Products in Human Epidermis. P. E. BOWDEN, P. T. BLADON, E. J. WOOD, AND *W. J. CUNLIFFE, Departments of Biochemistry and *Dermatology, University of Leeds, Leeds, U.K.

The multi-chain protein, keratin, is a major structural component of human epidermis. It is not synthesised directly but results from modification of the tonofilament protein, prekeratin, during terminal differentiation. Prekeratin also consists of several polypeptides and recent evident has shown that many of these are phosphorylated. The function of this phosphorylation is unclear; the purpose of this study is to investigate the molecular details of this post-translational event. Poly-adenylated messenger RNA isolated from scalp epidermis was translated in a reticulocyte lysate. Native prekeratin was labelled with either ^{35}S-methionine, ^{14}C-serine or ^{32}P-orthophosphate *in vitro* and isolated by acid extraction and isoelectric precipitation. Total translation products and labelled native prekeratin were compared by two-dimensional electrophoresis (IEF/SDS-PAGE) and fluorography. Phospho-amino acids were isolated and analysed by high-voltage electrophoresis. Native prekeratin consisted of 4 major polypeptides that contained phosphate bound covalently to serine. The isoelectric variants of the larger chains (mol.wt. 70 000 and 60 000) were neutral (pH 6.5–8.0) while the smaller chains were acidic (mol.wt. 57 000 and 52 000; pH 5.2–5.6). Serine and methionine labelled all these variants while ^{32}P-phosphate labelled all but the most basic variant. Furthermore, these non-phosphorylated basic variants co-electrophoresed in two-dimensions with major translation products of isolated epidermal messenger RNA. Therefore, it is concluded that in human scalp epidermis, there are at least four keratin gene products which demonstrate charge heterogeneity *in vivo*. This is due to post-translational phosphorylation of serine residues which may be a prerequisite for tonofilament assembly.

Production of Type III Collagen: Cell Free Synthesis and Regulation of pN-Propeptides. L. PHAN-THANH, R. TIMPL, P. MÜLLER, Max-Planck-Institut für Biochemie 8033 Martinsried bei München, BRD, T. KRIEG. Dermatologische Klinik der Ludwig-Maximilian-Universität 8 München, Frauenlobstr. 9–11, BRD.

The biomechanical properties of connective tissues are determined by their macromolecular structure, in particular by the presence of collagen fibrils, the formation of which is influenced by the type of collagen. The regulation of collagen type synthesis requires a stringent control, since failure may result in a connective tissue disorder such as osteogenesis imperfecta or Ehlers-Danlos syndrome type IV. In our present study we investigated the synthesis of type III collagen in cell strains deficient of type III (ED type IV) and cell strains with high production of type III collagen (Rhabdomyosarcoma). The cell were grown in monolayerculture in the presence of ^3H-proline. The synthesized collagen molecules were extracted and characterized by protein-chemical and immunochemical methods. The extracted m-RNA was enriched by affinity chromatography on oligo-dT-cellulose and sucrose gradient ultracentrifugation, and then translated into proteins using a reticulate lysate and ^{35}S-methionine with and without the addition of pN-propeptides. For both investigated cell strains similar patterns of collagens were found to be synthesized under cell culture conditions and in the cell free translation system. Furthermore type III collagen synthesis gradually decreased as the concentration of pN-propeptides increased providing further evidence for a regulatory role of the peptides, which act on a posttranscriptional level.

Inactivation of the Chemotactic Complement Split Product C5a by Psoralen-plus-UVA. JENS-M. SCHRÖDER, AND ENNO CHRISTOPHERS, Dept. of Dermatology, Univ. Kiel/W.-Germany.

Oral administration of 8-methoxypsoralen (8-MOP), followed by exposure to longwave ultraviolet-light (PUVA) results in clearing of generalized psoriasis. PUVA has been shown to inhibit epidermal DNA synthesis by producing interstrand crosslinks in DNA. In addition, during healing of the skin lesions polymorphonuclear neutrophil leucocytes (PMN) cease to migrate into the psoriatic epidermis. These cells are nonproliferative and therefore production of interstrand crosslinks does not seem to play an important role in the absence of these cells during PUVA therapy. Therefore we studied the *in vitro* effect of PUVA on the chemotactic activity of the C5a, which is supposed to be of importance *in vivo*. C5a was used after mixing with 8-MOP to a final concentration of 0.1 μg 8-MOP/ml. The mixture of C5a and 8-MOP was exposed to longwave UV. Controls contained 8-MOP and were not irradiated or contained no 8-MOP and were irradiated. Samples containing no 8-MOP and left unirradiated were analyzed in addition. The chemotactic activity of all samples was tested in a modified Boyden chamber system using normal PMN. Treatment of C5a with PUVA (0.1 μg 8-MOP, 1 J/cm^2 UVA led to a significant inhibition of C5a-chemotactic activity. The amount of inhibition showed a dependency on the C5a-concentration. These results demonstrate that PUVA is able to inactivate C5a. This may be for disappearance of PMN after PUVA of relevance. The mechanism of PUVA on C5a-inactivation is not yet known. We speculate that singlet oxygen, which can be produced by irradiation of 8-MOP, oxidizes methionin (position 70) which is essential for the chemotactic activity of C5a (amino acid 70).

Poster Discussion—E. FRENK, G. MICHAËLSSON, Chairpersons
6.30 PM, *E.S.D.R. General Assembly* (Active and Senior Members only)

Morning Session, Tuesday, April 6, 1982—*First Session,* 8:30–10:40 AM, R.A.J. EADY, CHAIRMAN

Ultrastructural and Biochemical Observations on the Effect of 4-Hydroxylanisole plus Tyrosinase on Normal Human Melanocytes and Keratocytes in Tissue Culture. S. PASSI, M. NAZZARO-PORRO, *A. BREATHNACH, E. ROBINS, L. ETHRIDGE.**, Inst Derm San Gallicano, Rome,* Dept Anat, St Mary's Hosp Med School, London.**

Mixed cultures of human melanocytes and keratocytes were exposed to 15 μg/ml of tyrosinase and 4-hydroxyanisole (40HA) at concentrations of 5×10^{-4}M to 5×10^{-2}M for periods up to 24 hr. No damage was suffered by either cell below 5×10^{-3}M 40HA for 6 hr, but higher concentrations and longer exposures produced extensive damage to both as observed ultrastructurally. Reversed-Phase High Performance Liquid Chromatography showed that toxic quinones are produced to a maximum 1 hr following the action of tyrosinase on 40HA, as is already known. Scanning spectrophotometry of mixtures of tyrosinase and 40HA in the presence of culture medium showed that whereas 40HA again acted as substrate for tyrosinase with the production of toxic quinones, these immediately disappeared, presumably due to reaction with nucleophilic substances in the medium; they would not therefore be available to damage melanocytes. Other substances with maxima of absorbance different to 40HA-quinone accumulated in the medium with time, and possibly are responsible for the nonspecific damage to melanocytes and keratocytes seen after 6 hr. The biochemical results can explain both the lack of specific effect of 40HA on melanocytes and the delayed nonspecific effect on melanocytes and keratocytes with the present cultures. There is still no explanation for the discrepancy between our results and those of Riley (*J Pathol,* 101:163, 1970) on cultured guineapig melanocytes, with an essentially similar medium.

Tyrosinase Assay in Highly Purified Cultures of Normal Guinea Pig and Human Melanocytes. J. AUBÖCK, M.D., D. KÖFLER, PH.D., M. SIFTER, P. FRITSCH, M.D. Department of Dermatology, University of Innsbruck, Austria, Anichstraße 35, A-6020 Innsbruck.

Biochemical research on normal mammalian melanocytes (M) has been hampered by the difficulties to establish pure M cultures and the impossibility to induce M proliferation *in vitro*. Based on a technique to prepare highly purified cultures of normal M (*Cell Biol Int Rep* 7:593, 1979) we present in this study a modification of the Pomerantz tyrosinase assay (*Biochem Biophys Res Commun* 16:188, 1964) applicable to the low numbers of M as can be grown in pure culture. Highly purified M cultures were prepared by plating primary epidermal cell suspensions from guinea pig ear epidermis in Mg^{++} free Eagle's medium into 25 ml Falcon flasks, resulting in 1–2.10^4M per flask. Tyrosinase activity (TA) was measured (1) from the amount of ^3H_2O released into the culture medium after incubation of living cells in situ with ^3H-tyrosine (1.0μCi/ml medium; 20 hr, 37°C) or (2) from the crude enzyme preparation obtained by treating the cells with 1% Triton-X 100 (60'; 20°C). With both methods, a basal TA per 10^4 cells were found in the range of 100–400% above the controls (culture medium without cells or incubate without cell homogenate). With both methods, pretreatment of the cultures with 10^{-6}M α-melanotropin (20 hrs, 37°C) resulted in increases of TA averaging 35%. Method (1) was considered superior to method (2) for its simplicity and for the avoidance of possible errors by the use of homogenates. To improve the ratio of cell number to the volume of incubation medium, the assay was carried out on suspended cells as a further modification: cells from a large number of flasks were harvested by trypsinization, pooled, and appropriate aliquots of cells kept in suspension with roller bottles, were incubated with ^3H-tyrosine. Under these conditions, however, the exposure to 10^{-6}M α-melanotropin did not produce any significant increase of TA above basal levels, presumably due to the removal of melanotropin receptors by trypsin. This latter modification was also employed on human M cultures: tyrosinase levels per 10^4 cells were found 300% above the controls.

Identical Lectin Labeling Profiles of Cultured Normal Human Melanocytes and Human Melanoma Cells. N. ROMANI, G. SCHULER, P. FRITSCH, Dept. Derm., Univ. Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

In primary epidermal cell cultures, guinea pig melanocytes (M) were shown to display specific lectin binding properties different from keratinocytes (K): M do not bind Soybean agglutinin (Ag) (SBA), Helix pomatia Ag (HPA) and Peanut Ag (PNA) due to masking of terminal D-galactose (D-gal) and N-acetyl-galactosamine (gal-NAc) residues, respectively, by sialic acid. The object of the present study was to determine if cultured human M conform to this rule, and if these cell surface properties are maintained after neoplastic transformation. Primary epidermal cell coverslip cultures were prepared from human split thickness skin according to routine techniques. Melanoma cell cultures were derived from lesions of 3 patients (2 lymph-node metastases, 1 superficial spreading melanoma). 4–6 day old unfixed cultures were incubated in situ (4°C, 20 min) with the following FITC and peroxidase conjugated lectins: Concanavalin A (Con A), Wheat germ Ag (WGA), SBA, HPA, PNA, Ulex

Europ. Ag I(UEA I) and Lotus tetrag.Ag(LTA). All lectins were tested, with and without prior neuraminidase treatment, by fluorescence and electron microscopy. Lectin binding profiles of normal human M and K were coincident to those reported for guinea pig epidermal cell cultures: Con A and WGA bound to both M and K; SBA, HPA and PNA bound to K but not to M; neuraminidase pretreatment, however, rendered M positive, indicating a masking effect by sialic acid. UEA I and LTA did not bind to M nor K; no change was induced by neuraminidase pretreatment. Ultrastructural findings were strictly coincident to those by fluorescent microscopy. Melanoma cells exhibited an identical labeling pattern to human M on both fluorescent and electron microscopic levels: as in M negative binding of SBA, HPA and PNA was rendered positive by neuraminidase pretreatment. We conclude that the masking of D-gal and gal-NAc residues by sialic acid is a stale property of melanocytic cells which may not be species specific and is maintained after neoplastic transformation.

The Role of Glycoconjugates in Keratinocyte Substratum Adhesion. G. P. ROBERTS, LUCY JENNER, AND R. MARKS. Department of Medicine, Welsh National School of Medicine, Heath Park Cardiff, CF4 4XN.

In a previous study we showed that a large number of glycoconjugates were synthesised in human epidermis on incubation of skin slices in the presence of radioactive precursors. A simpler system was required for the assessment of the role of these glycoconjugates in cell adhesion and consequently we have now examined the glycoconjugates synthesised by human keratinocytes in culture. Extraction of labelled keratinocytes with 0.5% Triton X-100 removed most of the glycoconjugates and left a substrate-attached material (SAM) which was then detached with 0.2% sodium dodecyl sulphate (SDS). Analysis of SAM by gel electrophoresis in the presence of SDS revealed the presence of material with the migration characteristics of hyaluronic acid together with seven large glycoproteins (molecular weights 232,000; 180,000; 162,000; 147,000; 126,000; 111,000, 97,000) and 5 glycoproteins in the keratin region (molecular weights 47,000 to 61,000). Trypsin digestion of labelled keratinocytes resulted in detachment of the cells and removal of most of the hyaluronic acid and large glycoproteins (molecular weights 97,000–232,00) but not the glycoproteins in the keratin region. Incubation of keratinocytes with tunicamycin did not cause detachment of the cells but did inhibit the synthesis of the glycoproteins with molecular weights 97,000–180,000. These results indicate that both the glycoprotein with a molecular weight of 232,000 and hyaluronic acid are involved in the adhesion of keratinocytes to the substratum.

Cultured Human Skin Fibroblasts and Keratinocytes: Regulation of Cholesterol Synthesis. M. PONEC, L. HAVEKES*, J. KEMPENAAR, B. J. VERMEER, Department of Dermatology, University Hospital and Gaubius Institute TNO*, Leiden, The Netherlands.

Cholesterol is an important constituent of cell membranes and disorders in metabolism of cholesterol can lead to serious disturbance in cell growth and function. It remains an open question whether there are substantial differences in the behaviour of particular cell types. In this study the regulation of cholesterol synthesis in cultured human skin fibroblasts and keratinocytes has been compared. The incorporation of ^{14}C-acetate or ^{14}C-octanoate into ^{14}C-cholesterol has been taken as a measure of the *de novo* cholesterol synthesis. The 2 types of cultured cells differed in the following features in the regulation of cholesterol synthesis (1) Keratinocytes synthesize a higher amount of cholesterol/mg cell protein (about 10 times). (2) Keratinocytes retain a higher amount of the de novo synthesized cholesterol, while fibroblasts release it to a much higher degree into the culture medium. (3) When extracellular environment is deprived of cholesterol the intracellular synthesis is markedly increased in fibroblasts while it stays practically unchanged in keratinocytes. (4) The low density lipoproteins (LDL) which enter the cells by receptor-mediated endocytosis and are degraded in lysosomes thereafter, liberate cholesterol which intervenes with the intracellular cholesterol synthesis. The lipoproteins suppress markedly the cholesterol synthesis in fibroblasts, but *do not* affect it in keratinocytes. (5) Also free cholesterol which is taken by the cells by passive diffusion reveals no effect on cholesterol synthesis in keratinocytes when added to the culture medium, in contrast to fibroblasts where a marked suppression is observed. (6) As compared with fibroblasts in keratinocytes the amount of ^{125}I-LDL bound specifically to the cell membrane receptor as well as the amount internalized and degraded by the cells is much lower. No binding of LDL to keratinocytes could be observed by ultrastructural studies.

Lecture, 10:00–10:40 AM—F. LEMBECK: "Neurogenic vasodilation in skin"

Second Session, 11:10 AM—1:00 PM—G. PROTA, Chairman

Chemotactic Properties and Biochemical Features of Malignant Cells In-Vitro.

H. MENSING,* A. Albini,[&] T. KRIEG,[§] P. MÜLLER,[&] B. PONTZ,[β] W. MEIGEL,[+]
[+]Univ.-Hautklinik 2000 Hamburg, [§]Univ.-Hautklinik 8000 München [&]Max Planck Institut f. Biochemie 8033 Martinsried/München [β]Univ.-Kinderklinik 6500 Mainz.

The pathophysiology of expansive growth and metastasis of malignant tumors is little understood. One of the crucial features of tumor-cells is their motility and response to chemoattractants like fibronectin (FN) and collagen derived peptides (CDP). Since tumor-cells are known to have an altered synthesis of collagen and FN resulting in a reduced connective tissue matrix it was the aim of this study to investigate their chemotactic response to FN and CDP. Rhabdomyosarcoma-, fibrosarcoma-, SV 40 transformed human lung fibroblasts and 5 cell strains derived from human dermatofibrosarcoma protuberans—each characterized by the synthesis of a specific pattern of structural proteins—were grown and compared to normal human fibroblasts in respect to their synthesis of collagen, FN and their chemotactic activity. In result chemotaxis was correlated to the amounts of FN synthesis of the different cell types: Cells, producing less FN show markedly increased chemotactic migration, when FN or CDP are used as attractant. In addition less minimal concentration of attractant was necessary to stimulate chemotaxis in these cells. In contrast no alteration of chemotactic activity could be noticed in DFP-cells, which show a collagen- and FNsynthesis similar to that of controls. These data suggest the idea that increased chemotactic response to relatively higher levels of FN and CDP in the environment of malignant tumors play an important role in the initial metastasizing process. In addition this could also explain the predominant location of metastases in connective tissue rich organs.

Five Years Observations on the Effect of Azelaic Acid on Lentigo Maligna. M. NAZZARO-PORRO, S. PASSI, G. ZINA*, A. S. BREATHNACH**, Institute of Dermatology St. Galicano, Rome.* Dermatological Clinic of the University, Turin, Italy. **Department of Anatomy, St. Mary's Hospital Medical School, London.

Since 1976 25 patients with Lentigo maligna were treated with topical (a 20% cream) azelaic acid for from 4 to 15 months. All cases showed positive results with progressive pigment loss up to complete clinical regression. During treatment, histology and electron microscopy confirmed reduction in number, massive lipid degeneration and destruction of atypical melanocytes, with return towards normal organisation of epidermis, reconstruction of basal lamina and disappearance of lymphocytic response. Dermal pigment within macrophages may persist for several months. The affected areas continue to appear normal up to 5, 4 and 2 yr in 4, 5, and 9 patients respectively, and at this time biopsy revealed normal cutaneous architecture and normal melanocytes. In 7 cases slight focal relapses developed; reapplication of cream resulted in complete healing of the lesion. No side effects of either toxic or allergic nature, and no local or focal hypopigmentation was observed. Clearly azelaic acid has a biological effect on pre-malignant melanocytes, possibly as competitive inhibitor of tyrosinase and/or by interfering with the mitochondrial respiratory chain. It may have a significant role in the treatment of Lentigo maligna, especially on the large lesions of the face and in old persons. The effect of azelaic acid on malignant melanocytes is under investigation.

Markers of Malignant Melanoma *In Vivo* and *In Vitro* and Localization of Melanoma-Associated Antigen p97 by Immuno Electron Microscopy.

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A melanoma cell line (MCL) was established and compared to biopsies and explant cultures to define properties which may be relevant to the management of melanomas (MM). Cells were examined by electron microscopy (EM), immunofluorescence (IF), protein analysis (gel electrophoresis GE), chromosome analysis (CH), flow cytometry (FC), immunohistochemistry (IH), and immuno electron microscopy (IM). Morphological variability was comparable *in vivo* and *in vitro*. Biopsies showed remarkable attachment plates between tumor cells (EM). Vimentin identified in cultured normal melanocytes (IF) was also detected as major cytoskeletal component in MM (GE) and in MCL (IF, GE). Analysis of MCL revealed a modal chromosome number of 56–58 with about 20% hypertetraploid metaphases and a superlong marker chromosome derived from No. 1 in 100% (CH). DNA-index was preserved in vitro with changes in phase fractions and ploidization. Consistent with CH a stable aneuploid stemline (2.6c) was identified in biopsy, explants and MCL (FC). Human melanoma-associated antigen p97 was detected in frozen sections of different melanomas at varying degrees also within the same melanoma by a monoclonal antibody (IH). In MCL the cell surface was heavily and continuously decorated with the antibody (IE). Since a positive reaction has also been shown in metastases this opens the possibility to detect early metastatic lesions by radioiodinated monoclonal antibodies. The feasibility of this approach has been demonstrated on a transplanted human melanoma in nude mice. The establishment of differentiation markers is of diagnostic value for identification and classification of malignant melanomas and may lead to clinical application in the treatment of malignant melanoma.

Cytokeratins of Human Skin Carcinomas. A. REANO, J. VIAC, J. THIVOLET.

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The cytokeratins of epithelial cells from basal cell carcinomas (8 cases) and squamous cell carcinomas (3 cases) free of surrounding normal tissue, were investigated for their biochemical and immunological properties. Cytokeratin proteins were extracted with high salt buffer and triton × 100 according to a technique described by Winter et al [1] and were comparatively analyzed by SDS polyacrylamide gel electrophoresis. Results showed in both tumors either the absence or a very low amount (5% of the total protein) of the major protein band of molecular weight (MW) 67 Kd present in normal human epidermis and confirmed immunolabelling results showing a staining limited to some dyskeratotic cells in cryostat sections of these tumors with an anti 67 Kd serum. In contrast gel electrophoresis showed in both tumors 3 distinct groups of predominant polypeptide bands of apparent relative MW values: (1) 60–65 Kd, (2) 52–55 Kd, and (3) 49 Kd representing respectively about 43%, 31% and 20% of the total proteins. Antibodies raised in animals against polypeptides 49 Kd and 52–55 Kd in a case of a large squamous cell carcinoma, strongly labelled in indirect immunofluorescence malignant cells present in these tumors. On normal human skin, the labelling was preferentially located to the cell cytoplasm of the basal and spinous layers of epidermis whereas anti 67 Kd serum labelled only the upper layers. These results confirmed the heterogeneity of cytokeratins expressed by human keratinocytes during the course of differentiation in epidermis. Although no "specific" keratin polypeptide seemed to be expressed in skin carcinomas, some major differences existed such as the relative increase of polypeptides of low MW and the very low amount of 67 Kd polypeptide. Such a result is in good correlation with the fact that cells with a mitotic capacity belong to the germinal layer.

1. Winter H, Schweizer J, Goerttler K: Keratins as markers of malignancy in mouse epidermal tumors. *Carcinogenesis* 1:391–398, 1980.

Lecture, 12:15–1:00 PM, D. DUMONDE: "Lymphokines"

Workshops, 1:00–3:00 PM. (1) Cytology of Inflammation, J. SONDERGAARD and L. DUBERTRET. (2) Porphyrins, F. DE MATTEIS and G. GOERZ. (3) Application of ultrastructural techniques to dermatology: recent advances, R. CAPUTO and G. SCHULER. (4) Retinoids, G. PLEWIG and F. FRITSCH.

Afternoon Session, 3:00–6:00 PM—*Poster Viewing,* 3:00–4:30 PM

Coated Langerhans Cell Granules in Histiocytosis-X Cells. G. SCHULER.

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Histiocytosis X cells (HXC) are closely related to epidermal Langerhans cells (LC) as they share morphological features and functional immunological markers (Fc-IgG, C3-receptors, and Ia antigens (*Arch Dermatol Res,* in press)). Although

LC granules (LCG) are the morphologic marker of LC and HXC, their significance and function is still unknown. Examining ultrathin sections of an eosinophilic granuloma we found that 5 to 10% of the LCG observed in the histiocytic cells—including those communicating with the extracellular space—to be surrounded by a fuzzy coat which consisted of periodically spaced bristles radiating from the cytoplasmic surface. This coat is indistinguishable from the clathrin coat of coated pits and vesicles and thus represents a strong morphological clue to the function of LCG. Clathrin coated structures are involved in diverse membrane translocation processes, notably receptor-specific endocytosis and intracellular protein traffic. Absence of a recognizable coat from most LCG of HXC as well as from LCG of normal LC may be either due to true absence of coat material (due to rapid depolymerization of the clathrin coat as has been shown to occur with coated vesicles during receptor specific endocytosis) or caused by presence of monomeric clathrin subunits not yet aggregated and thus invisible in routine sections. Immunoelectronmicroscopic studies using antibodies against reconstituted coat structures prepared from isolated coated vesicles are in progress to clarify this point.

Circulating Sézary Cells in Sézary Syndrome. WILHELM STOLZ, CHRISTIAN SCHMOECKEL, GÜNTER BURG, AND OTTO BRAUN-FALCO. Department of Dermatology, University of Munich, Frauenlobstr. 9, 8000 München 2, West Germany.

The diagnosis of Sézary syndrome (SS) also depends on the percentage of circulating Sézary cells (SC). However, their detection on blood smears appears difficult, particularly in the small cell variant. For this reason plastic-embedded buffy coats were studied by lightmicroscopy (LM, semithin sections) and by electronmicroscopy (EM, thin sections) in 9 SS (small cell variant), 4 widespread benign eczemas, and 3 healthy individuals. In each patient the nuclear contour index (NCI) of EM micrographs of 150 lymphoid cells was determined using Kontron MOP-AM03. These cells were classified by means of the NCI in 3 groups and correlated with the findings by LM: Normal lymphocytes (NCI $\leq$ 5.0), intermediate lymphocytes (5.0 $<$ NCI $\leq$ 6.5), SC (NCI $>$ 6.5). Furthermore the 25th and 70th percentiles of the NCI-values were calculated. The best distinction between SS and the controls was obtained by EM for 2 main reasons: (a) Sézary cells are not always revealed by LM. (b) The determination of the NCI is possible by EM only. When the following 3 EM criterias were fulfilled, all 9 SS were correctly classified: 1. Normal lymphocytes $<$ 63%. 2. Intermediate lymphocytes $>$ 20%. 3. Sézary cells $>$ 9%. In this way all controls were excluded. LM often appears to be sufficient for confirming the diagnosis of SS (small cell variant). For problem cases however, EM seems to be more conclusive.

A Specific Method of Langerhans Cells Quantification in Human Epidermis. Differences in Expression of HLA-DR & T6 Antigens. M. HAFTECK,[1] D. SCHMITT,[1] J. BROCHIER,[2] M. FAURE,[1] J. THIVOLET.[1] 1: INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot-Pav. R, 69374 LYON CEDEX 2, 2: INSERM U 80, Clinique Néphrologique, Hôpital E. Herriot-Pav. P, 69374 Lyon Cedex 2.

To facilitate LC number and distribution evaluation we propose a method of HLA-DR(+) and T6(+) cell quantification on frozen skin sections. Anti HLA-DR (Ia) and OKT6 immune sera are used as LC markers in an indirect IF method. The first reacts with monocytes, B lymphocytes, activated T lymphocytes and LC, the second-a monoclonal antithymocyte antibody-was found to be specific for intrathymic immature lymphocytes, skin "indeterminate" and Langerhans cells. Skin biopsies from forearm extensory surface of 10 healthy human subjects were examined. IF(+) cells' bodies were counted in epidermis on 4.5 μ cryocut sections under IF microscope equipped with an ocular meter grid covering 0.0576 mm2 of a specimen under 40 $\times$ magnification. At least 30 consecutive fields were examined and apart from number of IF(+) cells, also thickness of epidermis was noted. For each biopsy and each staining method we calculated: (1) mean cummulative number of IF(+) cells per 1mm2 of epidermal section surface-expressing relative IF(+) cells/keratinocytes ratio; (2) mean number of IF(+) cells per 1mm of epidermal section length-non dependent on epidermal thickness-informing on their surface distribution and surface density. Significant differences between T6(+) and HLA-DR(+) epidermal cells' numbers were found in normal human skin. Higher number of T6(+) cells than those expressing HLA-DR antigen may signify, that in normal conditions a part of dendritic cells reacting with OKT6 antibody do not possess Ia surface antigens, necessary for their immunologic functions. The proposed method proved efficient also in highly acanthotic or clubbed epidermis (conditions disableing LC quantification by other methods) simultaneously providing valuable insight at LC distribution, localisation and presence in a dermis.

Regional Differences in Normal Human Dermo-Epidermal Junction: A Morphometric Study of Ultrastructural Components. M.J. TIDMAN, R.A.J. EADY, D.B. GUNNER, A.R. KENNEDY, J.E.E. BRASSEY, AND M. F. CORBETT. Institute of Dermatology, Homerton Grove, London, England.

Quantitative data on the distribution of the structures comprising the normal dermo-epidermal junction (DEJ) would be of value as a baseline for the future investigation of such pathological processes as blistering and neoplasia. In the present study, skin from defined areas on the lower leg, thigh, and upper arm in 5 healthy men was processed for transmission electron microscopy, and electron micrograph montages (mag. $\times$ 44,500) of representative lengths of DEJ were compiled. The numbers of hemidesmosomes (HD) and plasmalemmal vesicles (PV) per 40μm of plasma membrane and of anchoring fibrils (AF) per 40μm of basal lamina were counted using a MOP image analysis system. The thicknesses of basal lamina (BL) and lamina lucida (LL), associated and unassociated with HD, were measured on sampled micrographs (mag. $\times$ 116,500), 6 readings of each measurement being made from each montage. A wide range of counts and measurements of DEJ components was found. HD counts ranged from 49–98 per 40μm and PV counts from 15–43. Analysis of variance showed that there was no statistical difference in HD and PV numbers between sites or subjects. Greatest variation was found in AF counts which ranged from 14 (arm) to 120 (thigh) per 40μm. There was a significantly lower number of AF in the upper arm compared with the 2 leg sites ($p < 0.01$). LL thickness varied from 11.23nm to 97.16nm with a mean of 49.52 $\pm$ 16.86 (SD) and BL thickness from 30.24nm to 156.82nm with a mean of 78.38 $\pm$ 21.54 (SD). HD-associated BL was thinner in skin from the arm compared with thigh and lower leg ($p < 0.05$). Further studies will assess the influence of age, sex and trauma on these parameters. Tests of dermo-epidermal adhesion using suction will be used to examine possible correlation with regional differences in AF.

I.E.M. Study of Langerhans Cells with O.K.T.6 Monoclonal Serum and Avidin-Biotin Amplification System. E. BERTI, M. MONTI, S. CAVICCHINI, F. PAROLINI, R. CAPUTO, 1st Clinic of Dermatology, University of Milan.

This work illustrates a method for the ultrastructural demonstration of Langerhans cells under normal and pathological conditions, using the OKT6 monoclonal antiserum and the avidin-biotin amplification system. 2×4mm fragments of healthy skin from normal subjects, of recents lesions of histiocytosis X and of erythemato-infiltrative lesions of mycosis fungoides were embedded in 7% agarose and fresh-cut with an agar-tissue-chopper. 40 μ sections were fixed in periodate-lysine-paraformaldehyde, pH 7.2, for 1 hr, washed for 15′×2 in PBS buffer, pH 7.2, incubated for 1 hr with OKT6 (ORTHOCLONE LOT. 11CO12), washed in PBS for 15′ × 2, incubated for 1 hr with biotinylated anti-mouse IgG (Vector Lab. Lot 01025), washed for 15′ × 2 in PBS, incubated for 30′ in horse radish peroxidase-avidin-D (Vector Lab. Lot. 01118), washed in PBS and then in TRIS-HCL, pH 7.6, incubated for 10′ in TRIS-HCL pH 7.6 + DAB (10 mg in 20 ml) + H_2O_2, fixed for 1 hr in 1% OsO_4 in Millonig buffer pH 7.2, dehydrated in alcohols and embedded in Epon according to traditional electronmicroscopy procedures. By this method, the plasma membrane of Langerhans cells only, under both normal and pathological conditions, shows uniform deposits of peroxidase, thus confirming the specificity of the OKT6 monoclonal serum. The Langerhans cells present in histiocytosis X thus appear to have membrane antigenic properties (revealed by OKT6) identical with those detected in healthy skin and mycosis fungoides. The method described is steadily reproducible, allows a good preservation of tissues and, thanks to the use of the avidin-biotin system, also allows a clear demonstration of the surface antigens of cellular plasma membranes.

Monocyte Phagocytosis Is Not Affected by Vindesine Administration. A. FATTOROSSI, Laboratory of Immunology, Aerospace Medical Center, Rome. S. MORETTI, Department of Dermatology II, University of Florence, Florence. A. PALERMO, Department of Dermatology II, University of Florence, Florence. B. GIANNOTTI, Department of Dermatology II, University of Florence, Florence.

Mononuclear phagocytes (MP) in 8 advanced melanoma patients and in 4 Kaposi's sarcoma patients all receiving Vindesine (VDS) 3 mg per m^2 i.v. bolus a week for 6 weeks were studied. Peripheral blood MP were prepared according to Bøyum, identified by means of NAE activity on cytocentrifuged smears and assayed for their ability to ingest IgG-coated latex (IgGL) via Fc receptor and non-IgG-coated latex (L); in parallel total and differential WBC count were performed (Giemsa stain) and MP scored on morphological criteria. The tests were repeated just before VDS administration, and after 24 hr and 48 hr during the 1st, during the 3rd and during the 6th week of the treatment. PMNs were also scored to evaluate the myelotoxic effects of VDS. In all experiments and irrespective of the severe drug induced myelodepression the percentage of MP identified by means of NAE activity paralleled the percentage of IgGL phagocytosing cells and both values were significantly higher ($p < 0.005$) than the percentage of L phagocytosing cells; the absolute number of MP identified via IgGL ingestion (and NAE activity) was always higher than the number of MP calculated on Giemsa stained smears. Since we have observed such relationships in healthy subjects it appears that neither tumor and/nor chemotherapy can induce significant modifications on phagocytic activity of peripheral blood MP. However, the absolute number of circulating MP was lower than in healthy subjects ($p < 0.01$) when assessed by IgGL phagocytosis (and NAE activity) or by Giemsa stained smears. Since these low values were present before VDS administration and did not vary during the treatment, one can assume that other factors, such as tumor dissemination, may be responsible.

Antioxidants Preserve the Ability of Macrophages to Generate Eosinophil Chemotactic Factor (ECF). BEATE M. CZARNETSKI AND MITHAT MARDIN, Universitäts-hautklinik, Münster and Bayer AG, Wuppertal, W. Germany.

ECF is a potent chemotactic factor for neutrophils, with a preferance for eosinophils. The factor is generated from macrophages, mast cells, neutrophils, and epidermal cells after action of a lipoxygenase on membrane arachidonic acid. During *in vitro* tissue culture for more than 24 hr, macrophages lose their ability to generate ECF during phagocytosis of zymosan or after stimulation with the ionophore A 23187. Possible reasons for this observation were explored in the present investigation. Change of the serum additives to the culture medium or their omission, as well as culture in Teflon bags instead of tissue culture flasks did not prevent the loss of the cells' ability to secrete ECF. Addition of new substrate (arachidonic acid, 1×10^{-4} to 5×10^{-7}) or of intermediates of ECF formation (5-HETE) was also ineffective, as was the addition of cyclooxygenase inhibitors such as indomethacin or aspirin. In the presence of reduced glutathione (10^{-3} M), or of the lipooxygenase inhibitors compound BW 755C (20 μg/ml) or nordihydroguaretic acid (10^{-5} M) during culture, cells retained their ability to secrete ECF on subsequent stimulation. These data suggest that special conditions must prevail at tissue sites for cells to continue to secrete ECF over extended periods and thus to maintain an acute inflammatory reaction.

Chemotactic Migration of Skin Fibroblasts Derived from Different Types of Mucopolysaccharidoses. B. PONTZ,† H. MENSING,[¶] P. MÜLLER,[&] M. CANTZ,[§] W. MEIGEL[¶], †UNIV.-KINDERKLINIK 6500 MAINZ, [¶]UNIV.HAUTKLINIK 2000 HAMBURG, [&]MAX PLANCK INST.F.BIOCHEMIE 8033 MARTINSRIED, [§]PATHOLOGISCHES INST. DER UNIVERSITÄT, HEIDELBERG.

The mucopolysaccharidoses (MPS) are rare genetic disorders of glycosaminoglycan metabolism. Patients with theses diseases accumulate excessive amounts of several mucopolysaccharides within the lysosomes of most tissues. In an *in vitro* study we examined the chemotactic property of skin fibroblasts from different types of MPS. In a second series of experiments we attempted to correct the chemotactic behaviour of these cells, by preincubation with conditioned medium (CM) from human embryo fibroblasts (HEF), which contains the lacking enzymes in each specific disease. Fibroblasts from MPS and controls were grown in a monolayer culture. As an attractant substance we applied CM of HEF. Cells were allowed to migrate for 4 hr at 37°C in a 5% CO_2 atmosphere. Results indicate that chemotactic response of all MPS cells tested was lower in comparison with controls especially for MPS type II. To correct the known enzyme defects, we treated confluent monolayer cultures with CM derived from HEF for 48 hr. After that chemotaxis was repeated. Now, better migration was noted except for MPS type II. Our investigation show that the storage of mucopolysaccharides in the different types of MPS is correlated with a change of another cell property, chemotactic migration. A partial restoration of chemotactic activity occurred after the addition of the lacking enzymes. Even after correction of the enzymatic defect in MPS type II, the cells do not obtain the control chemotactic capacity. This implies that in addition to increased storage of mucopolysaccharides other properties of the cells are affected.

Elemental Redistribution in Guinea-Pig Epidermis after Exposure to DNCB. M. LINDBERG, Res.ass., Department of Medical Biophysics, Karolinska Institute, S-104 01 Stockholm 60, Sweden. G. M. ROOMANS, PH.D., The Wenner-

Gren Institute, University of Stockholm, Norrtullsgatan 16, S-113 45 Stockholm, Sweden. B. Forslind, M.D. Ass. Prof., Department of Medical Biophysics, Karolinska Institute, S-104 01 Stockholm 60, Sweden.

The elemental content of the keratinocytes reveals information on the functional state of the cells, i.e., if the cells are capable to maintain the cellular Na/K homeostasis. In the present investigation we have used energy dispersive x-ray analysis to determine the elemental content in keratinocytes at different time intervals after exposure to a DNCB solution. In addition skin undergoing autolysis was studied with the same technique. Guinea-pig skin was clipped and subsequently exposed to a 10% DNCB solution in acetone for 1,3,6 and 24 hr. Nonexposed skin served as control. Biopsies were immediately frozen in liquid nitrogen, 10 m sections were cut at −20°C and freeze-dried. The elemental content in keratinocytes in the st. basale and in the upper st. spinosum was analysed with the electron probe. After 1 and 3 hr of DNCP exposure no significant changes in elemental composition were seen in the two epidermal layers studied. After 6 hours, however, marked changes had occurred. An approximately 50% decrease in P and K content and an increase in Ca concentration were seen. Mg, S and Cl were not significantly affected. The DNCB induced pattern of elemental redistribution differs qualitatively and quantitatively from that of autolysis (an increase of Na, Cl and Ca, a small decrease of K and approximately constant P and S). We propose DNCB to cause two effects on the keratinocytes: (1) inactivation of the membrane Na/K pumps, causing increase of Na and decrease of K (comparable to autolysis), (2) loss of P by an unknown mechanism and a concurrent efflux of Na and K could occur to balance the loss of negative charges. Diffusion of cellular components out of the keratinocytes could be facilitated by the oedema that occurs in irritant reactions.

A Novel System for the Air-exposed Culture of Human Epidermal Cells. Uwe Reichert and Yannick Jacques. Centre International de Recherches Dermatologiques (CIRD) Sophia Antipolis, F-06565 Valbonne.

With the usual *in vitro* cultivation techniques, keratinocytes do not express the complete pattern of terminal differentiation found in the living skin. Attempts, therefore, have been made to apply more organotypical conditions, i.e., to keep the cells exposed to air and with the nutritients passing from the dividing to the differentiated cells. We describe a novel emerged culture system that might be useful for the study of differentiation *in vitro*. Thin gelatin discs were swollen at 37°C under a shaking motion in 25 mM acetate buffer, pH 4.5, containing 0.5% glutaraldehyde. After 1 h, the cross-linking solution was replaced by 10 mM putrescine-dihydrochloride to inactive free aldehyde residues and to reintroduce new amino groups. The discs were placed on 0.5% agarose blocks and the whole system was equilibrated with growth medium. The gelatin was carefully overlayered with human plasma supplemented with 5 mg acid-soluble collagen/ml. Fibrinogenesis was induced by addition of 2–3 droplets of fetal calf serum. After overnight incubation at 37°C and washing with fresh medium, the fibrin-coated gelatin was seeded with epidermal cells (isolated from human foreskin or from a primary culture on collagen) and completely covered with growth medium. The behaviour of the cells could be observed with an inverted phase-contrast microscope. Whereas attachment and spreading of keratinocytes freshly isolated from skin was poor, a high plating efficiency could be obtained with cells from primary cultures. After having reached confluence, the medium above the cells was removed. In the emerged conditions, the keratinocytes continued to grow and stratified. The differentiated cells could be released by treatment with 0.3% trypsin and 2% EDTA (30 min at room temperature), whereas the basal cell layer remained closely attached to the gelatin. A comparative study of the differentiating process in immersed and emerged situations using histological and biochemical criteria is in progress.

A Skin Graft Model for Studying Full-Thickness Human Skin. J. N. Kearney,* G. Gowland,* K. T. Holland,† and W. J. Cunliffe,** University Departments of Immunology,* Microbiology and Dermatology, Leeds, U.K.

There is a need for a realistic model permitting the use of physical, chemical, or microbiological probes into human skin. We have developed a model for the transfer of full thickness human skin containing whole sebaceous follicles to fully immunocompetent hairless (hr/hr) mice. Graft rejection was suppressed using rabbit anti-mouse thymocyte globulin. This is the first time that a full-thickness xenograft of human skin has been reported. The potential of the model for studies on the microbiology of human skin has been assessed. The normal skin microflora of hairless mice differs from that of humans. High levels of aerobic coryneforms ($10^3 \cdot cm^{-2}$) and staphylococci ($50 \cdot cm^{-2}$) were observed, with lower levels of streptococci, bacilli and Gm -ve rods ($\simeq 5 \cdot cm^{-2}$); and the complete absence of *Propionibacterium spp.* Human grafts maintained their characteristic microflora throughout the observation period with *P. acnes* the dominant organism (10^4—$10^5 \cdot cm^{-2}$). There was a differential carriage of *Staphylococcus spp.* between the 2 skin types and no evidence of invasion of the graft by the murine microflora. A comparison of *P. acnes* density on skin before and at various times post-grafting revealed no significant temporal changes. This model should be useful for the study of human skin microbiology, particularly for detailed studies on *P. acnes* and is technically much simpler to maintain than previously reported athymic mouse models.

Evidence to Support the Mini-Pig as a Model for Human Skin Inflammation. A. Chatelus, A. Civier, * A. Fourtanier, C. Hensby, L. Juhlin, H. Schaefer. CIRD, Sophia Antipolis, Valbonne, France. * Sté L'Oréal, Department de Biologie, Aulnay sous Bois, France.

The requirement for a viable alternative animal model to reflect human skin inflammation has prompted us to examine the responses of mini-pig skin to 2 inflammatory stimuli, namely Trafuril and UV-B irradiation. Mini-pigs under general anaesthesia were used and abdominal hairs removed, where necessary, by clipping. A dry film containing 0, and 5% w/w Trafuril in a cellulose ester based vehicle was applied under occlusion for 1 hour. After removal of the film suction bullae were commenced and the resulting bullae sampled 2 h after the end of the application period. Irradiation of these animals with 3 MED UV-PB was also performed. Suction bullae were subsequently raised on control (non-irradiated) and irradiated sites at 2, 4 and 24 h after irradiation. The exudates were examined for PGD_2, $PGE_{2\alpha}$, and 6-oxo-$PGF_{1\alpha}$ by GC-MS. The results (ng ml^{-1} exudate mean—SEM) are summarized in the table and suggest a close similarity of PG response in mini-pigs to those observed in man and that the mini-pig might be a possible animal model for human skin pharmacology.

	PGD_2	$PGF_{2\alpha}$	6-oxo-$PGF_{1\alpha}$
Control	21.3	18.1	9.6
n = 12	±2.6	±1.4	±.5
Blank film	21.8	15.9	11.0
n = 12	±2.7	±2.7	±2.6
Trafuril 5% w/w in film	74.4****	56.8***	43.2***
n = 5	±10.7	±5.4	±.9
Hours after UV-B			
2 h	46.5**	31.6***	31.1***
n = 4	±7.6	±2.5	±4.3
4 h	53.6***	41.6***	28.9***
n = 4	+4.6	+4.4	+3.2
24 h	27.9	30.7*	14.3
n = 4	±8.8	±4.1	±1.8

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Characterization of MSH Peptides in Mammalian Skin. R. J. Penny, K. Ridley, R. Chalmers, A. J. Thody, and Sam Shuster, Dept. of Dermatology, University of Newcastle upon Tyne. NE1 4LP.

Many of the peptides found in the amphibian skin have homologues in the mammalian brain. We have examined the possibility that α-MSH, which is produced in the pituitary and brain of mammals, is also present in skin. Initial experiments showed α-MSH immunoreactivity to be present in rat (3.91 ± 0.62 ng/g N = 13) and human skin (6.62 ± 1.86 ng/g N = 15). We now examine the nature of this MSH and the possibility that skin can metabolism MSH. Human epidermis was homogenised in 0.1% (TFA), extracted with ODS silica (C18 Sep Pak) and subjected to HPLC (μ Bondapak C18 column, run in 27% acetonitrile/0.6% TFA at 2 ml/min). Fractions were assayed using a C-terminal α-MSH radioimmunoassay. Chopped rat skin that had been preincubated for 60 min at 37°C in Krebs Ringer bicarbonate was incubated with or without synthetic α-MSH. Medium was removed at various times, fractionated using HPLC and assayed. Epidermal skin extracts contained 4 peaks of immunoreactive C-terminal α-MSH. Two coeluted with mono- and des-acetyl MSH standards. An earlier peak was probably an oxidised MSH. A later running peak coeluted with the majorpeak of α-MSH in rat pituitary and was probably di-acetyl MSH. After incubation of rat skin alone, no immunoreactive α-MSH was found in the medium. Added α-MSH was rapidly degraded, and HPLC fractionation revealed the same 4 peaks of immunoreactivity. The presence of di-acetyl MSH, suggests that the skin is capable of acetylation reactions. The HPLC profile of these peptides is similar to that found in the pituitary. Whether the skin simply represents a site of clearance for circulating MSH peptides, or whether it activates circulating MSH peptides or produces them in situ is still not clear.

Photodynamic Cell Damage of Hematoporphyrin Derivative *In Vitro*. Terje Christensen,[x] Gunnar Volden,[xx] Johan Moan,[(x)] [x] Norsk Hydro's Institute for Cancer Research, Montebello, Oslo and [xx] Department of Dermatology, University of Tromsø.

Hematoporphurin derivative (HPD) is a photodynamically active agent giving a high remission rate of malignant tumors in combination with light. HPD does not bind to DNA which was why we tried to evaluate the damage on the subcellular level enzymatically. NHIK 3025 cells derived from a cervix carcinoma were irradiated with near ultraviolet light in the presence of HPD, and the release of lysosomal enzymes and LDH were determined.

Following a light dose causing no cell inactivation, a slightly inhibited enzyme leakage was seen the first 6 h followed by a slightly more release than from the controls later on. The enzyme activities of cell pellets made 24 h after exposure were 40–75% of control values due to either a small inhibition of cellular enzyme activity or of inhibited cell growth by this dose. A higher light dose inactivating 80–90% of the cells, caused a rapid release of both lysosomal and cytosol enzymes. The cell pellets contained very little of the enzymes 24 h after treatment and especially free intracellular enzymes had been released almost completely. This light dose resulted in the development of extensive vesicles on the cell surfaces. The enzyme pattern by using marker enzymes for different subcellular fractions suggest that the vesicles are derived from the plasma membrane and contain different cytoplasmic inclusions. Leupeptin, a protease inhibitor, did not protect the cells from inactivation or vesiculation indicating that proteolytic enzymes or their inhibitors working from outside the cells are of little or no importance for the photoinactivating process.

Photophysical, Photochemical and Photosensitizing Properties of E-4′-Methoxycinnamates. P. Morliere,° O. Avice,° M.T. sae Melo,°° L. Dubertret,° R. Santus.°° °Laboratoire de Dermatologie—Hopital Henri Mondor—94010 Creteil-France; °°Laboratoire Physico-Chimie de l'Adaptation Biologique—Museum National d'Histoire Naturelle—75231 Paris—France.

We have been studying *in vitro* photophysical, photochemical and photosensitizing properities of hydro and liposoluble molecules widely used as sunscreens: E-4′-methoxycinnamates. Under steady state irradiations (υ = 334 and 313 nm) an absorption decrease was observed for each compound. These changes were associated, as expected, with the formation of a single photoproduct, a Z isomer as prouved by NMR. Upon steady state irradiation, Z → E photoisomerization was competing with E → Z photoisomerization and a photostationary state was obtained (mixture of E and Z isomers), depending on molar extinction coefficients of each isomer and on E → Z and Z → E photoisomerization quantum yields. These quantum yields were determined in various solvents and in each cases values higher than 0.4 were obtained, showing the large efficiency of the isomerization. These quantum yields remained unchanged when irradiations were performed in O_2 saturated solutions. When the photostationary state is obtained, no absorption changes were observed for longer exposures, showing very low degradation quantum yields. Under aerobic conditions, no singlet oxygen production was observed. Time resolved laser flash spectroscopy (υ = 265 nm) did not show evidence for any observable intermediate and the E → Z photoisomerization was achieved in less than 50 ns. Then photoisomerization occurs either via an excited singlet state or via a very short lived excited triplet state. Thus E-4′-methoxycinnamates are unable to exhibit photosensitizing properities *in vitro*.

A Comparative Study of the Immunogenetics of Herpes Gestationis and Toxic Erythema of Pregnancy. R. C. Holmes, M. M. Black. Department of Dermatology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, England. D. C. O. James, J. Dann. The Anthony Nolan Laboratory, St Mary Abbot's Hospital, Marloes Road, London W8. England.

Twenty-six patients with immunologically proven herpes gestationis (HG) and 30 patients with toxic erythema of pregnancy (TEP) were HLA typed. We compared the HLA findings in the two groups and we investigated the relationship of HLA type to autoimmune phenomena and disease severity. There was a high frequency of the HLA antigens B8 (77%) and DR3 (81%) in HG compared with

their normal frequency in European causasoids (B8 16%, DR3 20%; Terasaki, 1980). The frequencies of these antigens were also increased by TEP (B8 27%, DR3 54%), though to a lesser extent. The most notable finding in HG was a very high frequency of the antigen combination DR3/DR4 (50%). This combination was present in only 1 patient with TEP and its normal frequency in European caucasoids is 4% (Terasaki, 1980). In accord with the high frequency of B8 and DR3 in HG we found that it was associated with autoimmune disorders; 5 patients had Graves' disease, 2 alopecia areata, 1 vitiligo, 1 ulcerative colitis and 43% of patients had thyroid autoantibodies. In contrast none of the patients with TEP had associated autoimmune disorders. Possession of the HLA antigen DR3 correlated with increased disease severity in both HG and TEP. The single patient with TEP who had the antigen combination DR3/DR4 had a particularly extensive and protracted eruption. The striking association of HG with the HLA genes B8 and DR3 and also thyroid autoimmunity supports its inclusion within the spectrum of autoimmune diseases. The genetic differences between HG and TEP support their classification as separate disorders. Our interpretation of their immunogenetic similarities is that, as both disorders are probably immunologically mediated, both will be susceptible to modulation by the immune response genes.

Terasaki PI (ed.): 1980. Histocompatibility Testing 1980. Copenhagen: Munksgaard.

Poster Discussion, K. MUSTAKALLIO, R. MACKIE, Chairpersons

Morning Session, Wednesday, April 7, 1982—*First Session,* 8:30–10:30 AM—G. STINGL, Chairman

The *In Vitro* Mixed Skin Cell Lymphocyte Culture Reaction (MSLR) in Man: Analysis of the Epidermal Cells and T Cells Subpopulations. J. CZERNIELEWSKI,[1] M. FAURE,[1] D. SCHMITT,[1] J. BROCHIER,[2] J. THIVOLET.[1] [1] INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R, 69374 Lyon, Cedex 2 (France); [2] INSERM U 80, Clinique Néphrologique—Pav. P, Hôpital E. Herriot, 69374 Lyon, Cedex 2 (France).

The ability of normal human epidermal cells (EC) to induce *in vitro* proliferation of allogeneic and autologous peripheral blood (PB) lymphocytes (PBL) was investigated using MSLR. EC and PBL were defined using specific monoclonal antibodies (MCAB): OKT3, OKT4, OKT8 for respectively T cells, Helper and Suppressor T subsets; OKT6 and HLA-DR AB for Langerhans cells. Responder (R) PBL and stimulator (S) EC from 10 healthy male adults, were cocultured (R:S ratio 1:1) for 5 days; PBL proliferation was measured by 3H-Thymidine uptake. MSLR were conducted with untreated cells as controls, and after either treatment of PBL with OKT3, OKT4 or OKT8+ complement (C′) (AB-C′ mediated cell lysis), or treatment of EC with OKT6 or anti HLA-DR + C′. EC stimulated vigorously allogeneic PBL (mean SI-stimulation index: ratio cpm MSLR/cmp L cultured alone − =16.3). Stimulation by autologous EC was lower (mean SI:4.2). Responses were abolished after treatment of PBL with OKT3+ C′, and with OKT4+ C′ or OKT8+ C′. Pretreatment of EC with anti HLA-DR+ C′ inhibited MSLR, while OKT6+ C′ only markedly reduced (mean SI:3.1). Results were related to enumeration by IF or trypan blue test after MCAB+ C′ cytotoxicity. Data show that PBT-OKT3+ cells proliferate *in vitro* in allogeneic and autologous MSLR; optimal responses depend on cooperation of OKT4+ and OKT8+ cells. EC defined by OKT6 or HLA-DR antigen are necessary. Differences in results using either OKT6 or anti HLA-DR cytotoxicity are discussed. (This work was supported by grant DGRST 80 7 0308).

Cutaneous Immunocompetent Cells Phenotype and Peripheral T Cells Subpopulations Ratio in Active Lichen Plan. M. A. GOMES, R. FERNANDEZ-BUSSY, D. SCHMITT, M. GAUCHERAND, G. MAUDUIT, J. THIVOLET. INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R, 69374 Lyon, Cedex 2 (France).

The purpose of the present study was to examine the phenotype of the cutaneous immunocompetent cells in lichen planus (LP) infiltrates and the peripheral blood T cells Helper/Suppressor ratios, by the use of monoclonal antibodies. 8 patients with active LP were studied, by indirect IF technique by the use of monoclonal antibodies directed against T cells populations (OKT3, OKT4, OKT8), Langerhans cells (OKT6, BL6) and HLA-DR antigens (BL2). The percentages of the different T cell subpopulations in the peripheral blood, were defined by indirect IF using OKT3, OKT4 and OKT8. The phenotype of the T cells of the dermal infiltrate was T3+, T4+, BL2+ and T3+, T8+, BL2+. OKT6, BL6+, BL2+ dendritic cells were present in great numbers in epidermis; in the biopsies from recent lesions, dendritic cells with the same phenotype were present in the dermis; in older lesions no OKT6+, BL6+ cells were identified in the dermis (Table). In 3 cases, the ratio between T4+ and T8+ lymphocytes were rather high. A study of other patients are in progress in view to establish if this increase is significant. Our results in the cutaneous studies suggest an immunological reaction including all the T cell subpopulations. A first stage of antigenic information may be mediated by Langerhans cells and helper cells. A second effector stage may be mediated by OKT3+, T8+ cells. Our results in peripheral blood studies supports the immunological phenomena suggested by cutaneous studies in recent LP lesions.

Modulation of Inflammatory Responses and Correction of T Cell Defects In Sarcoidosis with TP5. J. THIVOLET,[1] M. FAURE,[1] J. F. NICOLAS,[1] G. MAUDUIT,[1] A. CLAUDY.[2 1] INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R, 69374 Lyon, Cedex 2; [2] Hôpital Bellevue, Service de Dermatologie, 42 Saint Etienne.

TP5 is a synthetic pentapeptide (ARG-LYS-ASP-VAL-TYR) corresponding to thymopoietin 32–36, that induces maturation of thymocytes and has been shown to exhibit positive clinical effect in rheumatoid arthritis. TP5 was administered alone to volunteer adult patients with sarcoïdosis (S). TP5 was given IV, 50 mg, 3 times a week for 6 weeks to 3 patients with acute S:erythema nodosum (EN) bilateral hilar adenopathy, lung stage 1, fever; and 12 weeks to 6 patients with chronic cutaneous S (CS). Before start and every 3 weeks were noted: clinical features, ESR, routine laboratory tests, serum angiotensine converting enzyme; tests for cell mediated immunity (CMI): peripheral blood (PB) lymphocytes and T cells, Helper (H) and Suppressor (S) (monoclonal antibodies) T cells subsets counts, PB H/S ratios; *in vitro* T cell functions (LLT to PHA, Con A…); *in vitro* tests for CMI: Multitests with 7 different antigens including tuberculin; tests for humoral and auto immunity (PBB-cells; Ig levels; Immune complexes:autoantibodies); serum IgE levels and PMNS functions. EN disappeared within 3 weeks; hilar adenopathy more slowly (9 and 12 weeks after start). CS lesions decreased in size and infiltration in 6 or 9 weeks, in number more slowly in only 2 cases. No side effects were noted. No evident changes in routine tests, humoral, and autoimmunity IgE levels, PMNS functions were noted before and during TP5. Cutaneous anergy was observed in 9 cases before, and corrected with TP5; in patients with defects in PBT cells and Suppressor cells with high H/S ratios, a progressive normalization was noted, with return to normal of T cell balance and PBSuppressor cells levels. Clinical and immunological data support TP5 efficiency. (Work supported by Cilag-France aff. of Ortho P.C.)

Peripheral Blood T Cell Imbalance with T Cell and Suppressor T Cell Defects in Patients with Sarcoidosis. J. F. NICOLAS, M. FAURE, M. GAUCHERAND, J. CZERNIELEWSKI, J. THIVOLET. INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R 69374 Lyon, Cedex 2 (France).

Peripheral blood lymphocytes (PBL) from 16 patients with sarcoïdosis (S): 9 patients with skin sarcoids, (CS), patients with erythema nodosum (EN) and bilateral hilar adenopathy, and absence of responses to skin hypersensitivity reactions (Multitests), and 23 age-matched healthy controls were characterized by reactivity with monoclonal antibodies (MCAB) OKT3, OKT4, OKT8 directed to surface antigens of, respectively, T cells, Helper-Inducer (H) and Suppressor-Cytotoxic (S) T cell subsets. PBL were isolated by gradient density centrifugation, washed, suspended in RPMI 1640 + 5% FCS and 25 mM Hepes (5.10^6 cells/ml). 10 μl of each reconstituted AB were incubated with 200 μl of cell suspension, 30′, 4°C; cells were washed, incubated with 100 μl of FITC goat antimouse IgG AB in RPMI, 30′, 4°C, washed, resuspended, and % of fluorescent cells counted. In contrast to controls (% OKT3 = 69.7 ± 9; OKT4 = 54.76 ± 10.54; OKT8 = 41.80 ± 12.63) patients had low % of PB subsets (% respectively = 58.76 ± 14.03; 44.72 ± 13.42; 28.27 ± 9.81; $p < 0.02$). None had low total PBL counts. Among the 2 subgroups, only in patients with EN were these changes significative. The major decrease was that noted with OKT8+ cells (suppressor cells), with elevated OKT4/OKT8 cells (H/S) ratios: controls = 1.39 ± 0.36; CS = 1.63 ± 0.72 (NS) and EN 1.80 ± 0.47 ($p < 0.02$). Results are discussed in regards to observations of Suppressor T cell anomalies in S. They suggest a T cell subset imbalance with decreased levels of OKT8-Suppressor cells in patients with EN, but not in patients with CS.

T Cell Subpopulations of Cutaneous T Cell Lymphoma (CTCL). A. CHU, J. PATTERSON, C. BERGER, R. EDELSON. St. John's Hospital, London and Columbia Presbyterian Hospital, New York.

The T cell subpopulations in skin biopsies from 91 patients with CTCL and 19 with a variety of benign lymphocytic infiltrates were examined *in situ*. A series of monoclonal antibodies (OKT1-pan T cell, BE3-pan T cell, OKT4-helper, OKT8-suppressor, OKT6-Langerhans cell & thymocyte, OKT10-common thymocyte, OKI-HLA-DR) were used in direct and indirect immuno peroxidase reactions. In the CTCL patients 3 main patterns were observed: (1) 64% showed a homogeneous distribution of the different T cell subpopulations of which 60% (range 40–95) were OKT1+ and BE3+, 54% (30–95) OKT4+ and 8% (1–30) were OKT8+. Many showed an increase of OKT8+ cells up to 40–60% at the margins of the infiltrate. (2) 21% showed selective loss of OKT1 antigen, and 80% of these also showed loss of BE3. (3) 15% showed large numbers of OKT8+ cells (50–90) but the percentages of OKT1+ and OKT4+ were within the ranges seen in group 1, suggesting the presence of an immature population of T cell which are OKT4+, OKT8+. In 95% of the CTCL biopsies, 3–5% (1–10) OKT6+ cells were present in the dermal infiltrate and in 92%, 3% (1–20) OKT10+ cells were present. In 5%, the OKT10+ population increased to 60% in the lower margins of the infiltrate. In 90%, OKI+ cells were present in large numbers, 62% (30–95). In 56% epidermal involvement was observed. The majority of the epidermal cells were OKT1+, OKT4+, but in 26% OKT8+ cells were also seen. Comparing sections from CTCL and benign dermatoses the helpful differentiating features were (1) the homogeneous distribution of the T subpopulations, (2) selective loss of OKT1, (3) presence of immature T cells.

Defective *in Vitro* Reactivity of PB Lymphocytes to Stimulation by Epidermal Cells in Psoriasis. M. FAURE, J. CCZERNIELEWSKI, D. SCHMITT, J. THIVOLET. INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R, 69374 Lyon, Cedex 2 (France).

We investigated in the Mixed Skin Cell Lymphocyte Culture Reaction (MSLR) the ability of peripheral blood lymphocytes (PBL) from normal (N) or psoriatic subjects (P) to react *in vitro* to stimulation by autologous or allogeneic epidermal cells (EC) and the capacity of EC (NEC and PEC) to induce *in vitro* PBL proliferation. Stimulator EC were obtained through trypsinisation of skin specimen from control adult male volunteers (NEC), uninvolved (UEC) or involved (IEC) skin from 10 patients with acute, untreated psoriasis vulgaris. Responder PBL were obtained in controls (NPBL) and the same 10 psoriatic patients (PPBL). None had low PBL and T cell counts or PBT cells subsets imbalance. PBL and EC were cocultured in MSLR in RPMI 1640 + 10% inactivated AB serum, 2 mM-L glutamine and antibiotics, at 37°C, in a 5% CO2 + H2O air for 5 days. T cell proliferation was then measured by 18 hr 3H-Thymidine uptake. Stimulation index (SI) was evaluated by dividing the cpm in MSLR by the cpm of PBL cultured alone (cpm of EC alone:insignificant). NPBL proliferated in allogeneic MSLR in response to NEC. Stimulation by autologous EC was also observed, though weaker. In contrast, no stimulation was obtained in autologous MSLR with PPBL and PEC, either UEC or IEC. PPBL did not react to allogeneic stimulator NEC, or responses were lower than when using the same NEC and control allogeneic PBL. Responses of the same NPBL to stimulation by allogeneic NEC, UEC or IEC were similar. These data indicate that EC from controls and EC from psoriasis (UEC or IEC) may equally stimulate control PBL in MSLR, while PPBL show an abnormal reactivity to *in vitro* stimulation by EC, which correlates well previous observations of T cell dysfunctions in the disease.

Gliadin Antibodies in Bullous Pemphigoid and Dermatitis Herpetiformis. MARIANNE KIEFFER AND R. STC. BARNETSON, Addenbrookes Hospital, Cambridge and Royal Infirmary, Edinburgh.

We have measured IgG and IgA gliadin antibodies in the sera of 34 patients with bullous pemphigoid and compared them with those of 23 patients with dermatitis herpetiformis (DH) on a normal diet, who have previously been shown to have high titres of serum antibodies, 9 patients with pemphigus (foliaceus or vulgaris) and 23 controls. We used an enzyme-linked immunosorbent assay and found that not only do patients with DH have high titres of IgG and IgA gliadin antibodies in their serum but so do most patients with pemphigoid. This was not so in the pemphigus group or in the controls. None of the patients with bullous pemphigoid had evidence of clinical malabsorption, and previous studies of

pemphigoid patients have not demonstrated any jejunal abnormality. Eight of the patients with pemphigoid had high titres of antibodies to β lactoglobulin, but none had gastric, thyroid or reticulin auto-antibodies. The high titres of gliadin antibodies and β lactoglobulin antibodies could be explained if patients with pemphigoid have unduly permeable jejunal mucosa, and this requires to be ascertained. Sequential studies of patients with DH showed that of 10 patients who responded to a gluten-free diet with disappearance of their skin lesions, the titres of IgG and IgA antibodies fell in 7; this was not the case in 7 patients who did not respond to a gluten free diet. It is likely that those who did not respond to a gluten free diet were not sticking rigidly to their diet. The measurement of gliadin antibodies sequentially in DH patients might be helpful in ascertaining compliance.

Intermediate Filaments of the Vimentin and Prekeratin Type in Skin Cells of Man. G. MAHRLE, R. BOLLING, M. OSBORN, AND K. WEBER, Dermatologische Klinik und Max-Planck-Institut f. Biophys. Chemie, Göttingen, West Germany.

Recently, antibodies against intermediate filaments (IF) have been shown to allow differentiation of various cell types in cell cultures and animal tissue. In the present study antibodies against mesenchymal and endo-/ectodermal derived IF were applied to differentiate human skin cells by immunofluorescence.

Monospecific antibodies were raised in guinea pig against IF of the vimentin type (IFV) from mouse 3T3 cells and against IF of the prekeratin type (IFP) purified from cow snout.

In the dermis of normal skin fibroblasts and endothelial cells displayed IFV. In the epidermis IFV was observed in basal and supprabasal dendritic cells. In serial sections IFV containing suprabasal cells were consistent with anti-HLA-Dr positive cells. This and the distribution pattern of these cells in the epidermis were indicative for melanocytes and Langerhans cells. Dermal melanocytes in blue nevus and dermal nevus cells in nevocellular nevus did also stain. On the other hand, IFP was demonstrated in all epidermal cell layers including the basal and the horny layer, whereas the dermis did not reveal any IFP. In lichen planus, however, dermal hyaline or colloid bodies contained IFP supporting that they are of epidermal origin. In this study antibodies against IF are presented identifying keratinizing and nonkeratinizing cells in the skin and giving evidence to their ontogenic relationship.

Second Session, 11:00 AM–1:15 PM—H. H. WOLFF, Chairman

Announcement of Poster Awards and Closing Address by President

Biological Significance of the Replication of Epidermotropic Lymphoma Cells. G. E. PIERARD, C. FRANCHIMONT, AND CH. M. LAPIERE, Dept. of Dermatology, University of Liège, Belgium.

Epidermotropism of lymphoid cells is a character of early stages of most cutaneous T cell lymphoma (CTCL). Using standard microscopy and radioautography after *in vitro* and *in vivo* incorporation of tritiated thymidine we evaluated in 32 patients the replication of epitheliotropic lymphoid cells (ELC) in lesions of pagetoid reticulosis, parapsoriasis en plaques and follicular mucinosis as a function of the stage of CTCL. We observed 3 different grades in the replication of ELC. Grade 0: ELC, but no labelling and no mitosis. This is encountered in parapsoriasis en plaques and follicular mucinosis in absence of tumoral CTCL. Grade 1: ELC in S phase and in mitosis. This is encountered in localized pagetoid reticulosis. Grade 2: more than 10% of ELC in S phase but without mitosis in the epidermis. This is a characteristic feature of mycosis fungoides. It is also observed in lesion of parapsoriasis en plaques and follicular mucinosis present when tumoral CTCL is also present. Influence of the epidermis on CTCL evolution is heteregeneous when considering homing and proliferation of ELC. These 2 characters might depend on different control factors in cutaneous prelymphoma because they are dissociated (stage 0) or combined (stage 1). In stage 2, homing and proliferation of lymphoma cells are at random in the epithelial and dermis indicating most probably a loss of specific regulations by epidermis. This represents a prognostic factor for the evolution of CTCL.

X-Linked Ichthyosis—Patients and Carriers—Investigated by Steroid Sulphatase Activity in Leucocytes. W. P. DE GROOT, Dept. of Dermatology, Free University Hospital, de Boelelaan 1117, Amsterdam, The Netherlands. C. M. VAN DER LOOS, Dept. of Pathology, University Hospital "Wilhelmina Gasthuis," 1e Helmersstraat 104, Amsterdam, The Netherlands. A. C. JÖBSIS, Dept. of Pathology, University Hospital "Wilhelmina Gasthuis," 1e Helmerstraat 104, Amsterdam, The Netherlands.

The need for developing a practical method of diagnosing the X-linked steroid sulphatase deficiency syndrome existed since the available methods are complicated and time consuming.

We investigated steroid sulphatase activity directly in peripheral blood leucocytes from 14 healthy controls (6 males and 8 females) from 7 X-linked ichthyosis patients and 9 carriers, and from 6 patients with various autosomal recessive ichthyotic conditions. The leucocyte suspensions were diluted to a standardized protein concentration and subsequently treated with a detergent. After centrifugation, the clear supernatant was applied to a disc electrophoresis gel. The gel was then cut into slices and steroid sulphatase activity was assayed in each slice, using dehydroepiandesterone sulphate as a substrate. Leucocyte steroid sulphatase activity was measurable; the mean value of activity in the healthy females was 1.4 times the mean value in the healthy males. In patients with X-linked ichthyosis, virtually no activity could be measured. In carriers, a wide range of activity was found, varying from almost as low as the level in patients to the level found in normal females. Only in the 6 patients with autosomal recessive ichthyotic conditions the activity was within the normal range. (1) The firm diagnosis X-linked ichthyosis can be made by this method. (2) Values in carriers are in accordance with Lyon's hypothesis.

Sezary Cells Expression of HLA-DR Antigens. D. SCHMITT,[1] P. SOUTEYRAND,[1] J. BROCHIER,[2] J. THIVOLET. [1] INSERM U 209, Laboratoire de Recherche Dermatologique et Immunologie, Hôpital E. Herriot—Pav. R, 69374 Lyon, Cedex 2 (France). [2] INSERM U 80, Clinique Néphrologique, Hôpital E. Herriot—Pav. P, 69374 Lyon, Cedex 2 (France).

In 5 typical cases of Sézary syndrome (SS) with persistent high levels of circulating Sézary cells (SC), the expression of the HLA-DR antigens was studied in the blood and the skin using monoclonal anti HLA-DR antibodies. Positive and negative controls were performed respectively using monoclonal OKT3, OKT4 and OKT8,OKT6 antibodies. We report here the results of the quantitative study of the circulating SC in immunoelectronmicroscopy and of the immunocytological study of the SC involved in the cutaneous lesions. At ultrastructural level the circulating SC did not express the HLA-DR specificity and the phenotype of these cells was (T3+, T4+, T8−, T6−) HLA-DR-. On skin lesions, a large population of mononuclear HLA-DR positive cells was observed in all the cases. These cells appeared located in the upper dermis and in the epidermis. Some of them showed a dendritic appearance and could be Langerhans cells. The majority correspond to the lymphoid cells including the mononuclear phagocytes and a large proportion of tumoral SC. These results demonstrated the presence of a large population of HLA-DR positive tumoral SC in cutaneous lesions of Sézary syndrome. The HLA-DR specificity is expressed by monocytes, Langerhans cells, B lymphocytes and "activated" T lymphocytes. In skin lesions, the infiltrating SC, involved in the pathological process, expressed the DR specificity and seemed to be "activated," whereas they are DR negative in blood. Studies are in progress to precise the ultrastructural distribution of the DR antigens in cutaneous lesions of SS and to determine the possible role of these molecules in the keratinocyte-Langerhans cells-tumoral SC interactions in skin. (This work was supported by grant from DGRST (n° 80 7 03 08) and INSERM (CRL 81 10 28)).

MSH Control of Coat Colour in the Agouti Mouse. A. MCCORMACK, K. RIDLEY, R. J. CARTER, A. J. THODY, AND SAM SHUSTER, Dept. of Dermatology, University of Newcastle-upon-Tyne, U.K.

In order to study the role of MSH in mammalian pigmentation, we have studied its action, *in vivo*, on mouse coat colour. Changes in coat colour of the Agouti (C3H/He-A^{vy}) mouse were observed from birth to adult life and plasma immunoreactive α-MSH measured during this period. The effect of α-MSH administration and reduced circulating MSH (induced by CB154 implantation [0.83 mg/animal]) was studied. The mice were born hairless and grew a golden-yellow coat colour at 10–12 days. At 28 days, dark skin, due to the presence of pigmented hair, was observed, and over the next 6–7 days a dark grey pigmented coat grew, occurring first at the neck and proceeding as a wave towards the tail. Throughout this period, ventral skin remained pale. The coat colour changes were not related to circulating α-MSH levels and injection of α-MSH (5 μg/animal: sc, on alternate days for 10 days) had no effect on 10-day-old or adult mice. However, similar treatment of 22-day-old mice produced increased darkening of the coat at 28 days. Treatment with CB154 at 22 days inhibited pigmented hair growth at 28 days, but this was restored when α-MSH was administered together with CB154. We conclude that coat colour of the Agouti mouse is controlled by MSH, but only at the 22–28 day period. At this critical stage, melanocytes may become transiently sensitive to α-MSH.

UV-B Induced Unscheduled DNA Synthesis (UDS). Dose Response and Time Sequence in Human Skin. W. BRENNER, W. RAUSCHMEIER,[+] AND H. HÖNIGSMANN, Department of Dermatology (I), University of Vienna and Department of Dermatology, University of Innsbruck,[+] Austria.

UDS has been shown to be saturated above a threshold dose of UV-C in human fibroblasts and data from this laboratory have provided evidence that a similar saturation occurs in human skin *in vivo* after UV-B. This phenomenon was tested in detail by means of an expanded dose-response and time curve comprising a 24h period after exposure. In 17 subjects, a dose-response curve was established by exposure to 1/16; 1/8; 1/4; 1/2; 1; 2; 3; 4 and 6 MEDs UV-B. Shave biopsies were taken immediately thereafter. For the time curve study areas exposed to 1/2 and 2 MEDs were biopsied after 1, 3, 6, 12 and 24h. Autoradiography (^{3}HTdR, spec. act. 5Ci/mM) was performed *in vitro*. The dose-response curve showed a significant increase of both the SLC-indices (sparse-labelled cells/1,000 epidermal cells) and the grains/SLC from 1/16 to 1 MED (analysis of variance), whereas no significant difference was observed between 1 MED and the higher UV-B doses tested. Values of 1/2 MED differed only slightly from those of multiples of the MED. The 24h time sequence revealed a gradual decrease of UDS activity. The curves for SLC-indices and grains/SLC starting at essentially similar levels immediately after exposure (grains/SLC:1/2 MED 9, 8 ± 4, 4; 2 MEDs 9, 8 ± 5, 6; SLC-indices:1/2 MED 773 ± 33; 2 MEDs 764 ± 53) remained parallel during the first 3h. Thereafter the 1/2 MED curve declined more rapidly and reached the zero-level between 12 and 24h, whereas considerable UDS was still present 24h after 2 MEDs ($\bar{x}$ of grains/SLC:5, 1; $\bar{x}$ of SLC-indices:410). Preliminary results indicate that 24h after 2 MEDs low doses of UV-B suffice to saturate UDS capacity again. *Conclusions:* (1) UDS reaches a plateau after 1 MED and cannot be increased by doses up to 6 MEDs, suggesting a saturation of excision repair. (2) Time sequence studies support such a saturation phenomenon. (3) Because of the persistence of UDS 24h after 2 MEDs, a second hit of even suberythemogenic doses will induce a DNA damage sufficient to add up to plateau formation again.

Junctional Blisters in Dermolytic Bullous Diseases—Expression of the Role of the Lamina Lucida as Locus Minoris Resistentiae? G. KLEIN, H. HINTNER, G. SCHULER, P. FRITSCH. Dept. Derm., Univ. Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria.

Visualization of antigenic determinants of the dermoepidermal junction zone (type IV collagen = TIVC, laminin = La, bullous pemphigoid antigen = BPag) by immunofluorescence using BP serum and the respective specific antibodies, has been shown to permit rapid and accurate determination of the level of split formation in the disease spectrum of epidermolysis bullosa (*JID* 76:113, 1981). Dermolytic blisters are defined by the presence of TIVC (basal lamina) and La (lamina lucida, constantly linked to TIVC) in the blister roof; in junctional blisters, both these antigens are localized in the blister floor and only BPag in the blister roof. In this study we employed this technique on the following acquired bullous dermatoses: BP (n = 3), linear IgA dermatosis (LIGA) (n = 1), porphyria cutanea tarda (PCT) (n = 1) and dermatitis herpetiformis (DH; granular IgA) (n = 3). BP and LIGA are currently considered junctional, PCT and DH dermolytic bullous diseases. In BP and LIGA, all blisters were in fact of the junctional type. In PCT, 2 fresh blisters were—as expected—dermolytic; an older, larger blister, however, was frankly junctional. In DH, all blisters were clearly junctional although remnants of TIVC and La were detected at some spots of the blister roofs. All these findings were confirmed by electron microscopy. The occurrence of junctional bullae in dermolytic bullous diseases seems to militate against the current concept of a causal and constant relationship between the level of split formation and the nature of the respective disease. We suggest that junctional blisters may arise secondarily from dermolytic blisters subsequent to (mechanical?) damage of the basal lamina and spill-over of the blister fluid into the space of the lamina lucida. If this be correct, the lamina lucida can be viewed as a locus minoris resistentiae within the dermoepidermal junction zone.

Collagen Gene Expression in Fibroblast Populations Derived from Patients with Scleroderma. T. KRIEG, P. MÜLLER, C. LUDERSCHMIDT, S. PERLISH, R. FLEISCHMAJER. Dermatologische Klinik der LMU, München. MPI für Biochemie, Martinsried. Mount Sinai Medical School, New York.

Progressive systemic sclerosis (PSS) and localized sclerosis are diseases resulting in fibrosis of the skin. Since fibroblasts derived from patients with PSS have been reported to have a disturbed production of collagen it was the aim of our study to isolate different populations of cells from patients with active and nonactive forms of PSS and morphea. Furthermore we investigated whether cells producing excessive amounts of collagen in primary cultures keep this alteration during subcultures. Fibroblast cultures were established from skin biopsies. In some cases the dermis was dissected into different layers, cells were grown from each sample and investigated as primary cultures and after the 3rd and 5th passage. Following metabolic labelling with 3H-proline collagen production was estimated and characterization of the newly synthesized molecules were carried out by immunochemical and proteinchemical methods. Fibroblasts from patients in an active stage could be shown to produce excessive amounts of collagen. The most actively synthesizing cells resided in and were isolated from the subcutaneous fat. However no alteration of collagen type synthesis could be shown in those cultures. In some cultures the high level of collagen synthesis was seen as late as the 7th passage whereas in others this alteration was lost after the 1st trypsinization. These data provide evidence that only certain populations of fibroblasts in scleroderma patients are characterized by an excessive overproduction of collagen. However this alteration is then stable under culture conditions and even after several subcultures.

Cutaneous and Plasmatic Fibrinolytic Activity in Cutaneous Necrotizing Vasculitis. PAOLO FABBRI, GASTONE BIANCHINI, PIETRO CAPPUGI, PIERO CAMPOLMI, TORELLO LOTTI, Clinica Dermatologica Università di Firenze, Italia.

In their investigations of the role of the fibrinolytic system in cutaneous necrotizing vasculitis (CNV) some Authors have shown that the decreased lytic activity of fibrin by the physiological activators plays an important role in self perpetuating and amplifying the injury due to the etiologic factor. We have investigated cutaneous fibrinolytic activity in 50 subjects and plasmatic fibrinolytic activity in 20 subjects, all with CNV. Cutaneous fibrinolytic activity was investigated with Todd's autohistographic modified technique and plasmatic fibrinolytic activity with Fearnley's Euglobulin Lysis time test. Simple radial immunodiffusion for detecting plasmatic plasminogen and seric inhibitors of fibrinolysis (antithrombin III, α_2 macroglobulin, α_1 antitrypsin) was performed on all 50 subjects. Direct immunofluorescence for detecting fibrin antigen in vasculitic skin was also performed in all 50 cases. Our data show that fibrinolytic activity is decreased or absent in the affected skin or subjects with "palpable purpura," while in these same patients we observed an increased cutaneous fibrinolytic activity in the urticarial lesions sometimes present in the early stages of the disease. Direct immunofluorescence showed heavy deposits of fibrin-related antigen in the papular lesions and little or no deposits in the urticarial lesions. Circulating fibrinolytic activity was decreased in all 20 subjects investigated. Plasmatic plasminogen and seric AT III, α_2 macroglobulin and α_1 antitrypsin were normal.